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LIFE-HISTORY STUDIES OF THE TOBACCO FLEA-BEETLE IN THE SOUTHERN CIGAR-WRAPPER DISTRICT[1]

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INTRODUCTION

The tobacco flea-beetle[2] has been recognized for many years as a serious tobacco pest, especially in the tropical and semitropical tobacco-growing regions. Experiments and observations bearing on the control of this pest were carried on by the Bureau of Entomology at Quincy, Fla., from 1918 to 1923 (*2*).[3] In connection with this work a detailed life-history study in the southern cigar-wrapper district[4] was undertaken in 1920 and continued through 1923.

While a number of writers have discussed the life history of this insect, especially Chittenden (*3*, *4*), Howard (*5*), Morgan (*9*), and Metcalf (*7*, *8*), until the present time no detailed life-history records have been published.

REARING METHODS

Various means for obtaining eggs were tested during the course of this investigation. The method employed by Johannsen (*6*) in obtaining eggs of the potato flea-beetle was found to be the most satisfactory. The beetles are enclosed in a lantern globe, which is covered at each end with organdy and set in a vertical position over a flowerpot. The globe is placed upon a piece of black blotting paper kept damp by contact with moist earth in the flowerpot. The beetles thrust their ovipositors through the cloth and deposit their eggs on the paper, where they can be easily seen. In using this method it was found that a certain number of eggs invariably adhered to the meshes of the cloth. A count of the eggs was most easily obtained by using a dark-colored organdy on the end of the globe in contact with the paper.

To obtain incubation records, the deposition cages were placed on end in direct contact with firmed, moist soil contained in tin salve boxes, and the eggs were deposited directly on the surface of the soil. The larvae were reared in these boxes of earth over which large salve boxes were inverted. Food was supplied the larvae by placing sprouted tobacco seed on the surface of the earth. The moisture content of the earth was kept as uniform as possible by the occasional addition of a few drops of water.

Pupal records were most easily obtained by placing a piece of dark blotting paper over the earth in the boxes about the time that the larvae reached maturity. The pupal cells were formed in the earth directly beneath the blotting paper, where they could be easily observed. Although this work was performed on the laboratory porch, it was found that the soil temperature under these artificial conditions varied but slightly from the temperature of the soil beneath tobacco plants in the field, where the tobacco flea-beetle larvae and pupae normally exist.

DESCRIPTION OF STAGES

THE EGG

The egg (fig. 1) is elongate oval, slightly more pointed at one end, 0.362 to 0.483 mm. in length and 0.164 to 0.259 mm. in diameter, translucent and pearly white, gradually assuming a faint lemon-colored tinge as it grows older. Under the compound microscope the chorion apparently shows a slight but distinct reticulation.

THE LARVA

MATURE LARVA.—Larva delicate, threadlike, measuring about 3 mm. Color white, except on the chitinized parts, which are light brown; head capsule with dark margins and end of ninth abdominal segment blackish brown. Head small,

[1] Received for publication April 22, 1924—issued March, 1925.
[2] *Epitrix parvula* Fab.; order Coleoptra, family Chrysomelidae.
[3] Reference is made by number (italic) to "Literature cited," p. 584.
[4] Northern Florida and southern Georgia.

somewhat elongate and nutant. Labrum anteriorly rounded, with a row of short setae, two on each side. Clypeus transverse, also with a single row of small setae, three on each side. Frons distinct from epicranium; several short setae both on frons and epicranium. Ocelli absent. Antenna (fig. 3, B) short, attached to cranium by a large membrane (*m*), two-jointed; basal joint ring-shaped with two setae; apical joint minute, anteriorly with two horn-shaped prolongations (*h*); supplementary appendix (*a*) conspicuous, ovate. Mandible (fig. 3, D) ending with four smooth teeth, the two median ones falciform and larger than the two others; the proximal of the latter somewhat enlarged at base, the distal one slender and pointed; three long setae at inner margin near base and one long and strong seta on the external side of the mandible. Maxilla (fig. 3, C) with large and broad lobe (*l*), which on the inner margin carries a series of rather strong, stiff setae and at the end several smaller hairs in a ring around a papilla (*p*). Maxillary palpus conical, three-jointed, with terminal joint longer than the two others and medianly on the outer side carrying a single strong seta. Labium proper (*e*, fig. 3, C) transverse, posteriorly limited by a thinly chitinized arch; one short seta on each side. Labial palpus short, two-jointed. Ligula lacking. Mentum and submentum (*mt*) not separated, together forming a large, membranous, rounded region between the maxillae, with an anterior and posterior pair of setae. Prothorax with slightly chitinized tergal shield, the two other thoracic segments entirely fleshy. All thoracic segments sparsely provided with setae.

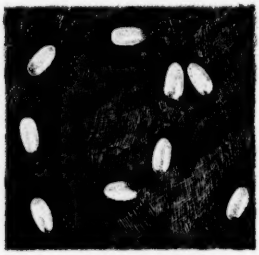

FIG. 1.—*Epitrix parvula*: Eggs. Greatly enlarged

Legs (fig. 3, A) short and rather weak; all equal and five-jointed; the fifth joint or "claw" hook-shaped, very curved and pointed; large membranous empodium (*e*) present.

First to eighth abdominal segments (fig. 3, F, H) cylindrical and separated by large intersegmental membranes (*i*). Dorsally each segment is divided into three folds, the anterior (*1*) carrying three short setae on each side, the second (*2*) one on each side, and the third (*3*) two on each side. Behind the spiracle (*s*) a single seta (*4*), placed in direct continuation of the two belonging to the third fold. Epipleural (*e*) and hypopleural (*h*) areas present, separated by distinct ventrolateral suture, and each carrying two short setae. Ventrally the segment has two transverse short folds (*v*, *w*) each with a few setae. At the base of all the setae are very inconspicuous, smooth and shiny, rounded, thinly chitinized plates.

Ninth abdominal segment (fig. 3, G), spatulate, posteriorly rounded; two pairs of long setae on its dorsal surface, the one pair (*1*) anteriorly, the second pair (*2*) posteriorly placed; laterally and posteriorly with three shorter but well developed setae on each side.

Tenth abdominal segment (fig. 3, E) retractile, cylindrical, and long, with the character of a locomotory organ.

Spiracles ring-shaped, small; one mesothoracic pair and eight abdominal pairs present.

LARVAL INSTARS.—First-instar larva 0.7 to 1.4 mm. long; white except the head, which is yellowish in color. Second-instar larva about 2 mm. long; white in color, with digestive tract showing darker. Last-instar larva about 3 mm. long; color white except on the chitinized parts, as the head capsule, which are light brownish.

THE PUPA

Uniformly white when first transformed. Length about 1.670 mm., width across mesothoracic femur about 0.959 mm. General appearance characteristic of the Chrysomelidae. Head bent downwards. Antennae directed caudad, about four of middle segments concealed beneath the prothoracic and mesothoracic legs, last three segments directed toward mesal line and lying posterior and nearly parallel to the tarsi of the mesothoracic legs. Metathoracic legs only partially concealed beneath elytra and wings, femur-tibial elbow approximately even with tip of elytra. Elytra overlapping fourth abdominal segment, wing almost attaining sixth segment of abdomen. Caudal segment of abdomen bearing a pair of characteristic hooklike appendages.

THE ADULT

The following description of *Epitrix parvula* is by W. S. Blatchley (*1*).

Oblong-oval, subconvex. Dull reddish-yellow elytra often with a fuscous transverse cloud at middle; abdomen brown; antennae and legs pale reddish-yellow, the four outer joints of former and hind femora of latter often darker.

Thorax convex, shining, nearly twice as wide as long, not narrowed in front; antebasal impression evident but not deep; surface distinctly but rather finely and sparsely punctate. Elytra very little wider than thorax, umbone feeble, punctures rather coarse and not crowded on disk; finer and more close-set on sides, where the intervals are subconvex. Length 1.5 to 2 mm.

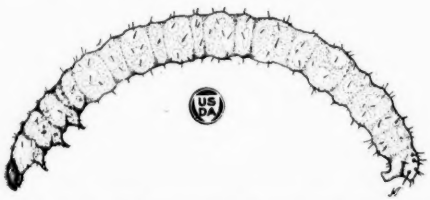

FIG. 2.—*Epitrix parvula*: Full-grown larva. × 17

LIFE HISTORY AND HABITS

LIFE-HISTORY SUMMARY

The eggs of the beetle are deposited in the soil near the base of the tobacco plant. They hatch in a few days and the larvae begin to feed on the small rootlets. The larval stage, which includes three instars, requires about 29 days at Quincy, Fla., during the cool weather of early spring, but in the hot weather of midsummer this period may be shortened by more than one-half. The pupal stage is passed in a small oval-shaped cell just below the surface of the soil. Newly emerged adults confine their feeding to the lower leaves

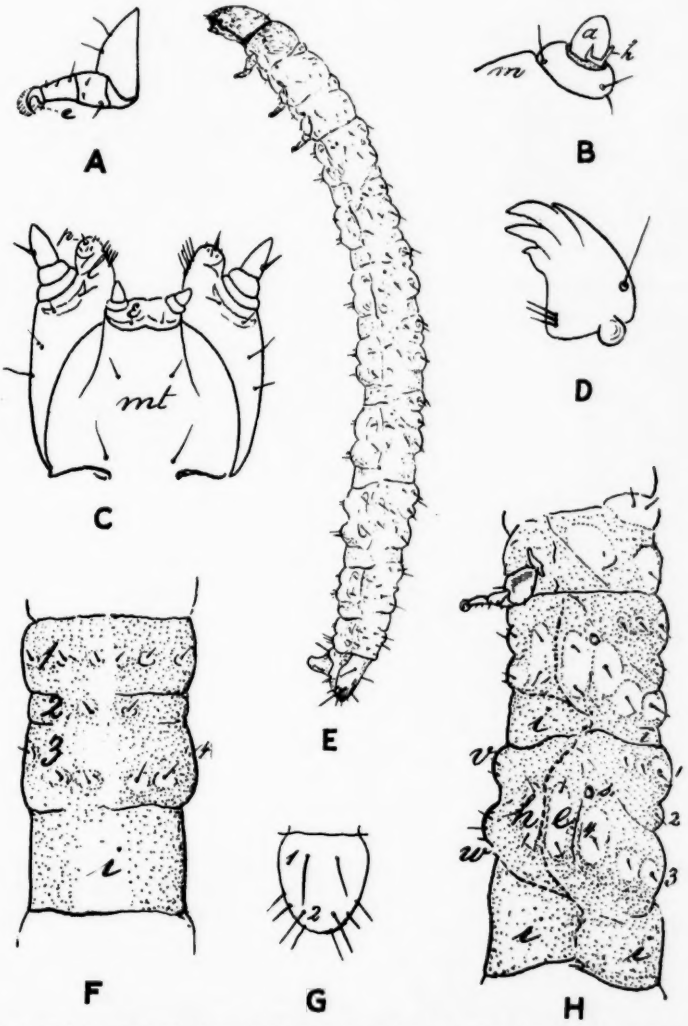

FIG. 3.—*Epitrix parvula:* Morphological details of mature larva. A, First thoracic leg: *e*, Empodium. B, Antenna: *m*, Basal membrane; *h*, apical joint; *a*, supplementary appendix. C, Ventral mouth parts *mt*, Mentum (=submentum); *e*, eulabium; *l*, lacinia; *p*, papilla. D, Left mandible, ventral view. E, Mature larva, lateral view. F, Second abdominal segment, dorsal view: *1*, First fold; *2*, second fold; *3*, third fold; *i*, intersegmental membrane. G, Ninth abdominal segment, dorsal view: *1*, Anterior long seta; *2*, posterior long seta. H, Third thoracic and first and second abdominal segments, lateral view: *i*, Intersegmental membrane; *s*, spiracle; *e*, epipleurum; *h*, hypopleurum; *w*, anterior ventral fold; *1*, anterior dorsal fold with three setae; *2*, median dorsal fold with one seta; *3*, posterior dorsal fold with two setae; *4*, single seta behind spiracle

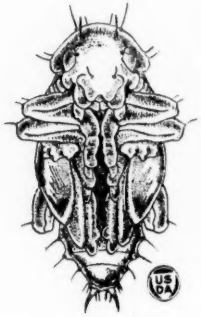

FIG. 4.—*Epitrix parvula:* Pupa. × 11

FIG. 5.—*Epitrix parvula:* Adult. × 20

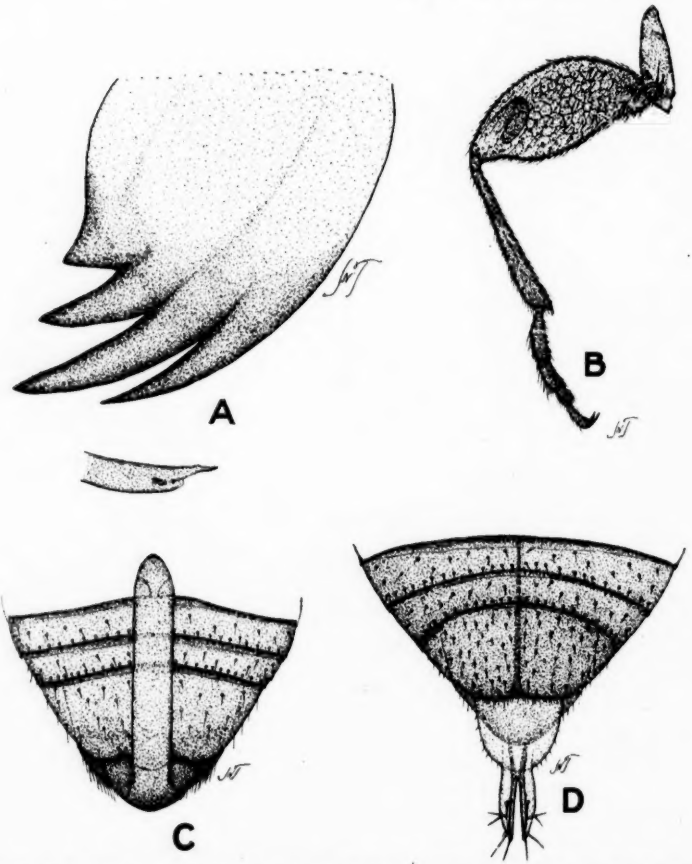

FIG. 6.—*Epitrigparvula:* A, Mandible of beetle. ×250; B, rear leg of beetle. ×58; C, tip of abdomen of male beetle showing genitalia extruded, with lateral view of tip of penis above. ×58; D, tip of abdomen of female beetle, showing ovipositor partially extruded, ventral view. ×58

which touch the ground, but older beetles feed on all portions of the foliage.

The tobacco crop is attacked by the overwintered generation and by two later generations of flea-beetles. After the crop is harvested there may be two and possibly three additional generations, which so overlap that it is impossible to tell them apart.

The winter is passed in the adult stage in a more or less incomplete state of hibernation.

THE EGG

HOW AND WHERE DEPOSITED.—The egg is deposited on or near the surface of the soil in cracks and crevices; usually with the point downward so that only the top of the larger end can be seen. It may be deposited, however, in almost any position; on end, lying on its side, or at an angle, depending more or less on the nature of the crevice in which it is placed. When tobacco is set out in the fields the eggs are usually laid in the moist depression about the base of the stalks where the plant is watered at setting time. Later in the season, when the plants are larger, most of the eggs seem to be deposited some little distance away from the stalk, beneath the lower leaves resting on the ground, where the beetles find more moisture and protection.

NUMBER OF EGGS DEPOSITED.—The number of eggs deposited by a single female appears to vary greatly; this variation doubtless is due to many factors. Under cage conditions, one lot of overwintered females confined with males laid an average of 164.47 eggs. Since the average longevity of the females in this cage was 44.8 days, an average of 3.67 eggs was deposited daily by each female. In this experiment, however, the beetles were not collected until the middle of April, and since oviposition in this locality frequently commences at a much earlier date, it is probable that a number of eggs were deposited before the flea-beetles were obtained. It can, therefore, be assumed that at least 200 eggs may be deposited by a female of this generation.

Two similar experiments with adults of the spring generation, in which a total of 9,257 eggs was deposited, gave an average deposition per female of 100.73 eggs. In both of these experiments the beetles used were collected within two or three days after emergence, so that these records may be taken as fairly indicative of the number of eggs deposited by the spring generation.

All records so far obtained indicate that the overwintered females deposit a much larger number of eggs than the females of the later generations. If this is correct, it is perhaps a provision of nature to counterbalance the heavy winter mortality.

Very few deposition records of individual females have been obtained by the authors. In one case, however, a single female deposited 12 eggs within 24 hours. Other records have been obtained of as high as 21 eggs deposited in a mass, more or less adhering together, all apparently having been deposited by one female.

TIME OF DAY WHEN OVIPOSITION OCCURS.—Eggs may be deposited at almost any hour of the day or night. The summarized deposition records shown in Table I indicate that the flea-beetles have no special time at which they deposit their eggs. In obtaining these records, which cover a period of 11 days, the deposition cage was placed throughout the day on the ground under a tobacco plant in the field, where a nearly normal condition of sunlight prevailed. During the night the cage was kept on the laboratory porch.

INFLUENCE OF SOIL MOISTURE ON DEPOSITION OF EGGS.—Soil moisture appears to have a considerable influence upon the deposition of eggs. Moist soil, when available, is always selected in preference to dry soil. In one case 350 flea-beetles, confined in a lantern-globe cage, were given an equal choice of either moist or air-dry soil upon which to oviposit. Over a 60-day period only 9 eggs were deposited on the dry soil as against 3,146 eggs deposited on the moist soil.

Where moist soil is unavailable upon which to oviposit the beetles deposit eggs very sparingly, even over long periods of time. Two deposition cages, each containing 150 flea-beetles, were started at the same time, the first being supplied with air-dry soil, the other with moist soil. Over a 60-day period 287 eggs were deposited on the dry soil as against 5,324 eggs deposited on the moist soil.

INFLUENCE OF TEMPERATURE UPON EGG DEPOSITION.—Deposition records of the tobacco flea-beetle indicate that no eggs are deposited when the daily mean temperature is below 50° F. No relationship between the number of eggs deposited and prevailing higher temperatures could be observed.

INFLUENCE OF TEMPERATURE ON LENGTH OF INCUBATION PERIOD.—Temperature seems to be the deciding factor in regulating the length of the incubation period. In the early spring

months at Quincy, Fla., incubation requires about 11 days, whereas in summer the eggs usually hatch in about 5 days. The period may, however, vary considerably within the same range of temperatures. Eggs deposited the same day sometimes vary as much as 4 days in the date of hatching.

TABLE I.—*Summarized deposition records of the tobacco flea-beetle obtained June 1 and 2, 1923, at Quincy, Fla., showing time of day at which eggs are deposited*

Time interval	Eggs deposited	Percentage
5 a. m. to 7 a. m.	130	9.0
7 a. m. to 12 m.	211	14.5
12 m. to 6.30 p. m.	366	25.2
6.30 p. m. to 8 p. m.	208	14.3
8 p. m. to 10.30 p. m.	244	16.8
10.30 p. m. to 5 a. m.	294	20.2
Total	1,453	100.0

other hand, gave as high a per cent hatch as in the case of soils of medium moisture content.

The greatest difficulty in life-history studies was encountered in the larval stage, owing primarily to the fact that the moisture requirements for this stage are so exacting. A certain amount of soil moisture is absolutely essential for larval development, but excessive moisture may prove to be very detrimental. On the other hand, the larvae seem to be able to develop successfully under a considerable range of temperature.

When maturity is reached the larva forms a small ovoid-shaped cell about an inch below the soil surface, and begins to shorten preparatory to pupation.

THE LARVA

DESCRIPTION OF LARVA EMERGING FROM EGG.—An egg under observation beneath the binocular microscope began hatching at 12.41 p. m. The larger end of the egg burst open as though

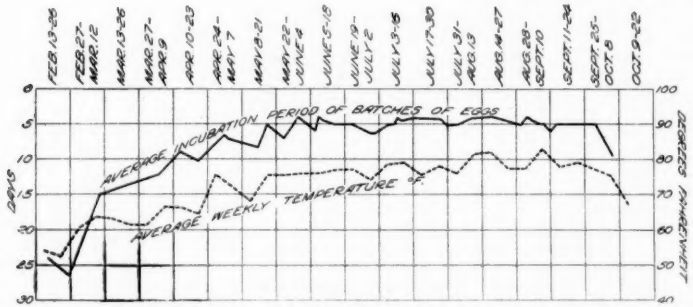

FIG. 7.—Relation of temperature to incubation of eggs of *Epitrix parvula*, Quincy, Fla., 1923

This effect of temperature upon the incubation of the tobacco flea-beetle egg is very marked. In one case, eggs deposited on February 12 required 26 to 27 days for hatching, whereas several records giving an incubation period as short as 3 days have been obtained in midsummer. The relationship between mean temperature and the length of the egg period is shown in Figure 7.

INFLUENCE OF MOISTURE ON INCUBATION.—The eggs of *E. parvula* require a certain amount of moisture for hatching, and the incubation period probably varies to a certain extent with the moisture conditions. Eggs placed by the beetles upon air-dry soil all failed to hatch, and eggs deposited on only slightly moistened soil gave a comparatively low hatch. Eggs deposited on water-saturated soil, on the

the larva had gnawed through from the inside. The head shield of the larva could be plainly seen. The head moved rapidly in a rotary manner, enlarging the opening, and was soon thrust out of the shell. After the head was free, movement ceased, except for a slow rhythmic movement of the body which could be plainly seen through the shell, and the larva rested for nearly a minute. Then the movement of the body quickened, the head moved rapidly from side to side, and in the course of about two minutes of unceasing exertion all of the prolegs were liberated. As soon as each proleg was freed it was brought into play. The larva twisted and pulled almost continuously, pausing for a second now and then, only to begin again with renewed efforts. At the end of exactly eight and one-half minutes from the

time the egg was ruptured, the anal segment was pulled clear of the shell, and the tiny larva crawled under the shelter of a large grain of sand to rest.

HABITS AND BEHAVIOR.—When first hatched, the tiny whitish larvae quickly start an active search for food. In their normal subterranean habitat they probably work their way through the loose soil, but in the covered rearing boxes of firmly packed earth, when food was available, they seldom burrowed below the surface.

The natural food of the tobacco flea-beetle larva is the tiny fibrous rootlets of the tobacco plant and other plants of the family Solanaceae. The young larva frequently bores into a root and tunnels it, but this habit, though common in the first instar, seems rare in the second and third instars. The older larva usually girdles a root or gnaws completely through it, severing it from the plant. No records of larvae feeding above ground have ever been obtained except under laboratory conditions. In the laboratory the best results were obtained by feeding with sprouted tobacco seed. Under these conditions several larvae have been observed to feed upon the small green cotyledonous leaves. Once the larva has begun feeding, it seldom moves about except in search of more food.

TABLE II.—*Length of instars of the tobacco flea-beetle (Epitrix parvula Fab.) at Quincy, Fla., 1922*

Date and time of day egg hatched	Date and time of day larva molted to second instar	Length of first instar	Date and time of day larva molted to third instar	Length of second instar	Date and time of day larva pupated	Length of third instar	Total length of larval stage
1922	1922	*Days*	1922	*Days*	1922	*Days*	*Days*
May 12, a. m.	May 20, a. m.	8	May 25, p. m.	5½	June 3, a. m.	8½	22
Do.	May 19, a. m.	7	May 22, m.	3¼	May 29, p. m.	7¼	17½
Do.	May 18, a. m.	6	May 21, m.	3¼	May 30, a. m.	8¾	18
May 12, m.	May 19, a. m.	6¾	May 23, a. m.	4	June 1, m.	9¼	20
May 13, a. m.	May 20, a. m.	7	May 24, a. m.	4	June 4, a. m.	11	22
Do.	May 19, a. m.	6	May 23, a. m.	4	May 30, p. m.	7½	17½
May 22, p. m.	May 27, p. m.	5	May 31, p. m.	4	June 8, a, m.	7½	16½
May 23, m.	May 28, m.	5	June 2, p. m.	5¼	June 9, a. m.	6½	16¾
Do.	May 28, a. m.	4¾	June 1, p. m.	4½	June 8, m.	6¾	16
Do.	May 28, p. m.	5¼	June 2, p. m.	5	June 8, a.. m.	5½	15¾
Do.	May 27, p. m.	4¼	June 1, p. m.	5	June 7, p. m.	6	15¼
Do.	May 28, a. m.	4¾	do.	4½	June 8, m.	6¾	16
Do.	May 28, p. m.	5¼	June 2, a. m.	4½	June 8, a. m.	6	15¾
Do.	May 27, p. m.	4¼	May 31, a. m.	3½	June 10, a. m.	10	17¾
Do.	do.	4¼	May 31, p. m.	4	June 7, a. m.	6½	14¾
Do.	May 28, a. m.	4¾	June 1, p. m.	4½	June 9, a. m.	7½	16¾
Do.	May 27, a. m.	3¾	May 30, p. m.	3½	June 7, a. m.	7½	14¾
Do.	May 28, a. m.	4¾	June 1, p. m.	4½	June 8, a. m.	6½	15¾
Do.	May 27, a. m.	3¾	May 31, a. m.	4	June 8, p. m.	8½	16¼
May 24, a. m.	May 28, a. m.	4	June 2, a. m.	5	June 9, a. m.	7	16
June 5, a. m.	June 11, a. m.	6	June 14, p. m.	3½	June 20, a. m.	5½	15
Do.	June 12, a. m.	7	June 16, a. m.	4	June 22, a. m.	6	17
Do.	do.	7	June 15, a. m.	3	June 23, a. m.	8	18
Do.	June 10, p. m.	5½	June 13, p. m.	3	June 21, a. m.	7½	16
Do.	June 12, a. m.	7	June 14, p. m.	2¼	June 20, a. m.	5½	15
Do.	June 10, p. m.	5½	June 13, a. m.	2½	do.	7	15
Do.	June 12, a. m.	7	June 14, a. m.	2	June 21, a. m.	7	16
Do.	June 10, p. m.	5½	do.	3¼	June 19, p. m.	5½	14½
Do.	June 11, a. m.	6	June 14, p. m.	3½	June 20, a. m.	5½	15
Do.	June 10, a. m.	5	June 13, a. m.	3	June 19, a. m.	6	14
Do.	June 10, p. m.	5½	June 14, a. m.	3½	June 19, p. m.	5½	14½
Do.	June 11, a. m.	6	June 15, a. m.	4	do.	4½	14½
Do.	June 11, p. m.	6½	June 14, a. m.	2½	do.	5½	14½
Do.	June 11, a. m.	6	June 13, a. m.	2	June 18, a. m.	5	13
Do.	do.	6	June 14, a. m.	3	June 19, a. m.	5	14
Do.	June 11, p. m.	6½	June 15, p. m.	4	June 24, a. m.	8½	19
Do.	do.	6½	June 14, a. m.	2½	June 20, p. m.	6½	15½
Do.	June 13, a. m.	8	June 16, p. m.	3½	June 25, a. m.	8½	20
June 16, a. m.	June 20, p. m.	4½	June 24, p. m.	4	June 29, m.	4¾	13¼
Do.	June 21, a. m.	5	June 24, a. m.	3	June 29, a. m.	5	13
Do.	do.	5	do.	3	June 30, p. m.	6½	14½
June 17, a. m.	June 22, a. m.	5	June 25, p. m.	3½	July 2, a. m.	6½	15
Do.	June 23, a. m.	6	do.	2½	do.	6½	15
June 29, a. m.	July 3, p. m.	4½	July 8, a. m.	4½	July 16, p. m.	8½	17½
Do.	July 5, a. m.	6	July 9, a. m.	4	July 15, a. m.	6	16
June 30, a. m.	July 4, a. m.	4	July 8, a. m.	4	July 14, a. m.	6	14
Do.	July 3, a. m.	3	July 7, a. m.	4	July 12, a. m.	5	12
Do.	July 4, a. m.	4	do.	3	July 13, a. m.	6	13
Do.	July 6, a. m.	6	July 9, p. m.	3½	July 15, p. m.	6	15½
Do.	July 4, a. m.	4	July 7, p. m.	3½	July 12, p. m.	5	12½

Total number of larvae, 50; average length of first instar, 5.48 days; average length of second instar, 3.67 days; average length of third instar, 6.70 days; average length of larval stage, 15.85 days.

DURATION OF THE LARVAL STAGE.—The length of the larval stage seems to depend mainly upon the temperature conditions. While in midsummer the stage may not require more than 11 days, in the early spring records of periods as long as 41 days have sometimes been obtained. Records obtained at Quincy, Fla., May 12 to July 16, 1922, are concisely set forth in Table II, which shows for each of 50 larvae the relative length of the three instars and the complete larval stage.

THE PUPA

When first transformed, the pupa is white in color and of the same approximate size as the beetle. During the resting stage the development can be plainly traced and the approximate age of the pupa readily determined. In summer the stages of development are as follows: First day, white all over; second day, compound eyes apparently dotted all over with reddish-brown; third day, all of compound eyes reddish-brown and tips of mandibles beginning to show a slight reddish color; fourth day, eyes almost black, tips of mandibles a dark reddish-brown, femur-tibial joint of each leg yellowish-brown, tarsi of legs very light brown, and entire body has taken on a dirty grayish appearance with the exception of the abdomen, which is still white; fifth day, parts showing color on the fourth day gradually darken until imago appears.

The length of the pupal stage is much shorter than that of the larval stage, but is also dependent to a certain extent upon temperature, and probably moisture, conditions. Pupal records obtained at Quincy, Fla., at various seasons of the year show a duration of from 4 to 11 days. The average length of this stage during the summer is approximately 5 days. The pupal record of 40 individuals taken at Quincy, Fla., May 28 to July 21, 1922, is shown in Table III.

TABLE III.—*Length of pupal stage of the tobacco flea-beetle at Quincy, Fla., May 28 to July 21, 1922*

Date of pupation	Date adult appeared	Length of pupal stage
		Days
May 28, p. m.	June 4, p. m.	7
May 29, p. m.	June 5, p. m.	7
May 30, a. m.	do	6½
June 3, a. m.	June 8, p. m.	5½
June 7, a. m.	June 12, a. m.	5
Do	do	5
Do	June 12, p. m.	5
June 8, a. m.	do	4½
Do	June 13, a. m.	5
June 8, m.	do	4¾
Do	do	4¾
June 8, p. m.	do	4½
June 9, a. m.	do	4
Do	do	4
Do	June 13, p. m.	4½
June 10, a. m.	June 14, a. m.	4
June 18, a. m.	June 23, a. m.	5
June 19, a. m.	June 24, a. m.	5
Do	June 24, p. m.	5½
June 19, p. m.	June 24, a. m.	4½
Do	June 24, m.	4¾
Do	do	4¾
Do	June 25, a. m.	5½
June 20, a. m.	do	5
Do	do	5
Do	do	5
June 20, p. m.	June 26, a. m.	5½
Do	do	5½
June 21, a. m.	June 25, p. m.	4½
Do	June 26, a. m.	5
June 29, a. m.	July 4, a. m.	5
June 30, p. m.	July 5, p. m.	5
July 2, a. m.	July 7, a. m.	5
July 12, a. m.	July 16, p. m.	4½
Do	July 17, a. m.	5
July 12, p. m.	July 18, a. m.	5½
July 13, a. m.	July 18, p. m.	5½
July 13, p. m.	do	5
Do	July 19, a. m.	5½
July 14, a. m.	July 21, a. m.	7
Average of 40 records of length of pupal stage.		5.11

THE ADULT

FEEDING HABITS.—When transformation to the adult stage has taken place, the beetles lie inactive within their cells from one to two days. Emergence then takes place, after which they rest for a period of about 24 hours. For several days after emergence the young beetles can be distinguished from those of the older generation by the fact that they are lighter and brighter in color and smaller in size. When the resting period has been completed, they commence feeding on the lower tobacco leaves which rest on the ground. In most of this early feeding the beetles confine their eating to the lower leaf surface. In a short time, however, the feeding punctures go entirely through the leaf.

The beetles remain on the lower leaves for three or four days and then gradually spread over the entire plant. In the case of unshaded tobacco the feeding is largely confined to the lower portion of the plant. In the case of shaded tobacco, however, the upper leaves are frequently punctured to a considerable extent and even the bud leaves are sometimes fed upon. Under shade conditions there seems to be little choice between the upper and lower surfaces of the leaves, although in high tobacco there is apparently some slight preference for the upper surface. Numerous observations have shown that the beetles feed at night as well as during the day.

While tobacco is the preferred food plant of the tobacco flea-beetle, many other plants, wild and cultivated, belonging to the Solanaceae are fed upon to a considerable extent. In the absence of solanaceous plants, the beetles will feed sparingly upon various other wild and cultivated plants.

PREOVIPOSITION PERIOD.—On May 8, 1921, two adults emerged from pupal cells and were immediately transferred to an oviposition cage. On May 23 the first eggs were deposited, giving a preoviposition period of 15 days. Again, on June 25, 1922, six tobacco flea-beetles, which had just emerged from their pupal cells, were placed in an oviposition cage. No eggs were deposited in this cage until July 17, an interval of 22 days. Other experiments also bear out the fact that there is a period of about two to three weeks after emergence from the pupal cell before eggs are deposited.

OVIPOSITION PERIOD.—Deposition of eggs seems to begin very soon after emergence from hibernation. Indeed, if the weather is mild there are indications that a few scattering eggs may be deposited through the winter. At least one record has been obtained of eggs deposited on February 3.

From the middle of March to late summer deposition is fairly constant. The number of eggs laid may fluctuate more or less, judging from the appearance of the different generations, but since to a certain extent the generations invariably overlap, some eggs are always being deposited. After the tobacco is harvested, however, flea-beetles gradually become very scarce. There seems to be little activity in late summer or early fall, and after the beginning of September the deposition of eggs seems nearly to cease.

PROPORTION OF SEXES.—Of 407 overwintered beetles collected in early spring at Quincy, Fla., in 1922 and 1923, 68 per cent were females and 32 per cent males. In 1922, of 500 beetles of the spring generation collected in the field, 65 per cent were females and 35 per cent males. Of 500 beetles of the first summer generation collected during the same season, 52 per cent were females and 48 per cent males. These data show a predominance of females over males, especially in the overwintering and the spring generations.

MATING.—Mating was commonly observed at all hours of the day. Just how much time elapses between emergence of the adult from the pupal cell and the act of mating is not known, but it is probable that mating occurs during the first week.

Although several attempts were made, no eggs were obtained from unfertilized females.

DISPERSION.—The tobacco flea-beetle utilizes all three of the common methods of locomotion — crawling, jumping, and flying. Perhaps most of the movement from plant to plant in the field is done by the first two of these methods, but the flea-beetle is quite capable of sustained flight. Seed-beds have been known to become infested with this pest when the bed was located in the midst of heavy timber a mile from where any tobacco had been planted previously. When a seed-bed is located near a tobacco shade, beetles migrate readily from the bed to the shade. Numerous records have been made of the collection of this beetle on fly-paper screens 5 and 6 feet above the ground. It is scarcely conceivable that the tobacco flea-beetle should be capable of flying many miles, but that they can and do fly several hundred yards is unquestionable.

LONGEVITY.—Just how long a tobacco flea-beetle may live under normal conditions is largely a matter of conjecture. In the field the overwintered beetles have largely disappeared by the end of April. With the exception of the overwintering individuals, however, the average length of life under field conditions appears to range approximately from 40 to 50 days. Laboratory records obtained over a period of several years indicate that the average length of life in confinement somewhat closely follows that in the field.

Several records have been obtained of a few flea-beetles living for an abnormally long time. In one case where 200 overwintered beetles were placed in confinement April 1, the last beetle died August 14. Of another lot of adults placed in confinement about the middle of March, the last individual died July 5. In a third instance, 200 freshly-emerged adults of the spring generation were placed in confinement April 17. The last individual died September 25. This beetle had a definitely known longevity of 161 days. These records show that it is possible for a few individuals of one generation to live so long that their lives overlap two following generations.

NUMBER OF GENERATIONS.—There are usually four fairly well-defined generations in the region of Quincy, Fla.: The overwintering generation, which generally appears about the time tobacco plants are up in the seed-beds;

the spring generation, which emerges from about May 1 to May 15, when tobacco is from 12 to 18 inches high; the first summer generation, which appears from the middle to the end of June, when tobacco is nearly mature; and a second summer generation, which may appear upon late tobacco during the latter part of July or the early part of August.

In addition to the four broods mentioned, there are probably one or two more partial generations, but their numbers are so few and scattered, and the preceding generations so overlap, that it is impossible to make a definite statement concerning them.

HIBERNATION.—The tobacco flea-beetle passes the winter in the adult stage. In northern Florida this period is passed in an incomplete state of hibernation. During cold weather the beetles seek shelter under piles of leaves, weeds, trash, or any kind of material which affords protection. During warm periods, however, they may become active and are often to be found feeding in sheltered spots. During unusually warm weather breeding may take place to a limited extent.

SUMMARY

The tobacco crop in the southern cigar-wrapper district is attacked by the overwintered brood and by two later generations of the tobacco flea-beetle, *Epitrix parvula*. After the crop is harvested there may be two and possibly three additional broods, which so overlap that it is impossible to tell them apart.

The eggs of this beetle are usually deposited in cracks or crevices in the soil near the base of the plant.

Females of the overwintered brood may lay as many as 200 eggs, but the deposition in later broods is much less.

Moist soil, when available, is always selected for deposition in preference to dry soil. When moist soil is unavailable, the beetles deposit eggs very sparingly, even over long periods of time.

Egg deposition, as well as feeding, may take place at any time during the day or night.

In the early spring months at Quincy, Fla., incubation requires about 11 days, whereas in summer the eggs usually hatch in about 5 days. A certain amount of moisture is necessary for incubation.

The larvae of the tobacco flea-beetle live underground and feed upon the roots of the tobacco plant and other plants of the family Solanaceae.

Larval records obtained at Quincy, Fla., show that the length of this stage may vary from 11 days in midsummer to 41 days in early spring.

The pupal stage is passed in a small oval-shaped cell about an inch below the surface of the ground. The average length of this stage during the summer is about five days.

Newly-emerged flea-beetles remain near the ground and confine their feeding to the lower tobacco leaves. In a short time, however, they spread over the entire plant and feed on all portions of the foliage.

While tobacco is the preferred food plant of the tobacco flea-beetle, other plants, both wild and cultivated, belonging to the Solanaceae are fed upon to a considerable extent. In the absence of solanaceous plants the beetles will feed sparingly upon various other wild and cultivated plants.

Deposition of eggs commences soon after emergence of the beetle from hibernation and continues until early fall.

The tobacco flea-beetle is capable of flying a considerable distance, this being the most important means of dispersion.

Hibernation in the southern cigar-wrapper district is incomplete.

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THE DIFFERENTIATION OF PRIMARY ISOLATIONS OF BACTERIUM MELITENSIS FROM PRIMARY ISOLATIONS OF BACTERIUM ABORTUS (BOVINE) BY THEIR CULTURAL AND ATMOSPHERIC REQUIREMENTS[1]

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INTRODUCTION

That the resemblance of *Bacterium abortus* to *Bact. melitensis* in morphological, cultural, biochemical, and serological respects is so close that their differentiation is difficult if not wellnigh impossible was discovered by Alice C. Evans (*6*)[2] in 1918, and later confirmed by Feusier and Meyer (*8*). This has led investigators to wonder whether the likeness in the two organisms ceases with form, action, and appearance upon artificial culture media and serological characteristics, or involves pathogenicity as well. Investigations bearing upon pathogenicity made later by Fleischner, Vecki, Shaw, and Meyer (*9*) indicate that the similarity even with respect to pathogenicity may be less remote than formerly suspected. Further information on this phase of the subject has been contributed by Khaled (*18*), Huddleson (*16*), Evans (*7*), and others.

In view of the fact, however, that severe illness in humans has never been definitely traced to the ingestion of *Bacterium abortus* in milk, few investigators have considered the two organisms identical; and the practice of utilizing milk containing this microorganism is far too general to permit ignoring the great quantity of available evidence thereon. Comparative studies of *Bact. abortus* and *Bact. melitensis* seem to have been mainly confined to strains which have been under artificial cultivation for considerable periods of time and are well accustomed to propagation upon artificial media rather than to those of recent isolation.

During 1922 and 1923 the writer had opportunity to make comparisons of some recently isolated strains of the two types of organisms. Results of this work seem to indicate that by cultural methods *Bacterium melitensis* and *Bact. abortus* (bovine) may be readily distinguished during their primary isolations.

The cultural results differed but little from those anticipated. Investigators of Malta fever have written little if at all of special or unusual biological requirements of the Malta fever organism, whereas in bovine infectious abortion the peculiar cultural characteristics of *Bacterium abortus* seem to have been the factor which retarded its discovery. The originality manifested by Bang and Stribolt (*1*) in their successful cultural work with *Bact. abortus* has never ceased to receive the profound respect which it rightfully deserves.

EXPERIMENTS AND RESULTS

During August, 1922, 24 samples of blood serum from goats suspected of being affected with Malta fever were forwarded to the Pathological Division, Bureau of Animal Industry, by Dr. W. A. Heck, a practicing veterinarian of Donna, Hidalgo County, Tex. When the samples were subjected to the agglutination test for this disease, four reacted in a dilution of 1 to 1,000, one 1 to 500, seven 1 to 200, two 1 to 100, one 1 to 50, and one 1 to 25. Eight gave negative results to the test in a dilution of 1 to 25 or higher. About a month before these samples arrived, T. B. Hooks, of Donna, Tex. (owner of the animals), had inquired if any test could determine whether the goats were affected with Malta fever. He stated in the communication that his brother, who had been using milk from this flock, had been suffering six months with some kind of fever suspected of being Malta

[1] Received for publication June 3, 1924; issued March, 1925.
[2] Reference is made by number (italic) to "Literature cited," pp. 590-591.

fever. The animals were described as fine Nubian milk goats costing from $200 to $600 each and the flock was mentioned as consisting of about 100 head. Sterile containers were shortly afterward forwarded to Doctor Heck with the request that he obtain from some of the higher reacting goats specimens of blood, milk, and urine for cultural and inoculation work. The reactors had been destroyed before the request was received; consequently no further action was taken at that time.

About two months later, W. E. Whigham, of Donna, Tex., wrote asking permission to forward for the application of the agglutination test for Malta fever blood-serum samples from two flocks of goats, approximately 100 head each, which had been exposed to the flock previously tested. Doctor Whigham stated that he had had three or four cases of Malta fever in human beings contracted from drinking the milk of these goats, one of his patients being the owner of one of the exposed flocks.

ORIGIN AND METHOD OF ISOLATING BACTERIUM MELITENSIS

On November 23 and 25, 212 more serum samples from the two flocks mentioned were received. Of this number three reacted to the agglutination test for Malta fever in a dilution of 1 to 1,000, one 1 to 500, nine 1 to 200, eleven 1 to 100, seven 1 to 50, and eight 1 to 25. The remaining samples were negative in dilutions of 1 to 25 or higher.

On November 6 a sample of human blood was received from Doctor Whigham. It was described as being obtained from one of his patients, a Mexican goat herder, 14 years of age, who had reacted to the Malta-fever test. The boy had been suffering with fever about 9 months, more or less continuously, his temperature reaching 102 to 103° F. in the afternoon. The specimen was somewhat decomposed upon arrival, but was sown on serum agar, subjected to the agglutination test both with a *Bacterium melitensis* and *Bact. abortus* antigen, and used for animal-inoculation purposes. The sample reacted in a dilution of 1 to 1,000 with both antigens.

In making the inoculations 11 guinea pigs were used; 6 were injected intra-abdominally with a physiological salt solution suspension of the blood and 5 both intra-abdominally and intratesticularly. Intratesticular inoculations had been described by Meyer and his coworkers (*21*) as an effective way of transmitting the disease through injections of *Bacterium melitensis* cultures. On December 22, between five and six weeks from the date of inoculation, five guinea pigs were destroyed. Two had received the material into both the abdomen and one testicle, and three had received it free in the abdominal cavity. Blood serum from these animals gave negative results to the agglutination test for Malta fever. Serum-agar slants sown with the spleen tissue remained sterile.

On December 28 the six remaining guinea pigs were destroyed. The autopsy findings and the cultural and serological results were as follows:

Guinea pig No. 77012. Inoculated intra-abdominally and intratesticularly. Spleen was somewhat thickened and surface irregular. Mesenteric lymph glands were congested. The inoculated testicles showed a constriction about midway between its extremities. On one side of the constriction was a small abscess containing semifluid pus. Other organs appeared normal. Cultures were made from the heart, liver, spleen, testicles, kidneys, and mesenteric lymph glands. Serum-agar slants alone were used. Blood serum reacted to the agglutination test with both *Bacterium abortus* and *Bact. melitensis* antigens in a dilution of 1 to 1,000. Its end point was not determined.

A portion of the cultures from each organ was incubated in closed jars in which 10 per cent of the air had been displaced by CO_2 gas and in which a ball of moistened cotton had been placed, providing conditions highly favorable for the growth of *Bacterium abortus*. Another lot was incubated under normal atmospheric conditions. The tubes in all cases were merely closed with cotton plugs, and the incubator was maintained at a temperature of 37° C. On December 30 the cultures were examined with no evidence of growth. On January 2, two cultures from spleen incubated under CO_2 conditions showed from 100 to 200 small opalescent colonies, two from affected testicle 15 to 20 colonies so small as to be recognized with difficulty, two from mesenteric lymph glands 10 to 15 colonies, one from kidney sterile, one from liver 3 colonies, one from heart sterile. Two cultures from spleen incubated under normal atmospheric conditions showed 200 to 300 colonies somewhat larger than those developing in a 10 per cent CO_2 atmosphere, one tube from testicle 50 to 75 colonies, one from liver 40 to 50

colonies, one from heart sterile. The organisms seem to have multiplied somewhat more rapidly in all cases under normal atmospheric conditions than in air partially displaced by CO_2 gas.

Guinea pig No. 77013. Inoculated both intra-abdominally and intra-testicularly The inoculated testicle showed a slightly abnormal appearance, probably at site of injection. An area about one-fourth inch in diameter was darker in color than the surrounding tissue. No other lesions were noted. The blood serum of this pig showed a titer of 1 to 50 with a *Bacterium melitensis* antigen and below 1 to 25 with a *Bact. abortus* antigen. Spleen and affected testicle cultured. Cultures were subjected to the same conditions as those of guinea pig No. 77012. On January 2, two cultures incubated under CO_2 conditions from spleen showed from 100 to 200 colonies; two tubes from testicle were sterile. The colonies developing under normal atmospheric conditions were slightly larger than those incubated in a 10 per cent CO_2 atmosphere.

Guinea pig No. 77014, inoculated into both testicle and abdominal cavity, and guinea pigs Nos. 77016 and 77107, which received the material intra-abdominally only, showed no lesions. Their blood serum did not agglutinate suspensions of either *Bact. abortus* or *Bact. melitensis* in dilutions of 1 to 25 or higher. Cultural results were also negative.

The colonies obtained in cultures from the two positive guinea pigs Nos. 77012 and 77013 closely resembled those of *Bacterium abortus*, but failed to attain, in four days, the size commonly reached by such colonies on serum-agar slants when incubated in moist jars containing 10 per cent CO_2 gas. A few of the larger colonies were approximately one-sixteenth inch in diameter. In most cases they were considerably smaller. On January 16 subcultures were made from the original spleen and testicle cultures on 3 per cent glycerin-agar slants. Slight growth was visible on the day after incubation under normal atmospheric conditions. On the second day the growth was reasonably heavy. Antigens prepared from subcultures of the organisms isolated from guinea pigs Nos. 77012 and 77013 were agglutinated in a characteristic manner by *Bact. abortus* and *Bact. melitensis* antiserums.

Efforts failed to isolate *Bacterium melitensi* from the blood of the 14-year-old boy by cultural methods. The serum-agar slants sown with different dilutions of the blood were heavily overgrown with contaminating organisms within a few hours.

On December 14, through the kindness of Doctor Heck, blood specimens were obtained from four of the goats, Nos. 96, 26, 61, and 19, exposed to the original reacting flock. Milk samples were also secured from three of these. The agglutination titer of the blood serums of goats No. 96, 26, and 61 with a *Bacterium melitensis* antigen was 1 to 1,000; goat 19, 1 to 500. The milk whey of goat No. 96 was negative; goat No. 26, titer 1 to 1,000; goat No. 19, titer 1 to 100.

Different dilutions of the blood and milk were sown on serum-agar slants and incubated under CO_2 and normal atmospheric conditions. On some of the tubes there developed colonies bearing rather close resemblance to those of *Bacterium abortus* and *Bact. melitensis;* but subcultures of these failed to produce antigens that were agglutinated by *Bact. abortus* and *Bact. melitensis* antiserums.

Four guinea pigs received 2 c. c. of milk each from three of the goats and the same number received suspensions of the blood of each blood sample. The milk was somewhat putrid on arrival. All the guinea pigs receiving the milk of goats Nos. 19 and 96 were dead in less than 48 hours.

Guinea pig No. 76595, inoculated with the blood of goat No. 61, died December 22. Cultures from spleen, liver, and kidneys remained sterile.

Guinea pig No. 77411, inoculated with the blood of goat No. 26, died December 30. Cultures from the liver and spleen remained sterile.

Guinea pig No. 77496, inoculated with milk from goat No. 26, died December 31. Cultures from liver and spleen developed colonies of *Bacillus coli;* no *Bacterium abortus*-like colonies were detected.

Guinea pig No. 77497, inoculated with the milk of goat No. 26, was destroyed January 29. Spleen was three times normal size. Liver showed evidence of fatty degeneration. Titer of blood serum to *Bacterium melitensis* antigen was 1 to 200; to *Bact. abortus* antigen, 1 to 200.

Guinea pig No. 77498, inoculated with the milk of goat No. 26, was destroyed January 29. Spleen was slightly enlarged. Right epididymis showed evidence of necrosis. Titer of blood serum to both *Bacterium melitensis* and *Bact. abortus* antigens was 1 to 500. Serum-agar slants, 3 per cent glycerin-agar slants, and infusion bouillon were used for culturing the spleens and testicles of guinea pigs Nos. 77497 and 77498.

COMPARISON OF CULTURAL AND ATMOSPHERIC REQUIREMENTS OF BACTERIUM ABORTUS (BOVINE) WITH THOSE OF BACTERIUM MELITENSIS

When the isolation of *Bacterium melitensis* through guinea-pig inoculations were being attempted, the writer was also testing the efficacy of abortion-bacterin treatment as a means of eliminating *Bact. abortus* from udder-infected cows. Sixteen cows, all cases of natural infection, were being used in this experiment. Three or four guinea pigs were injected with 5 c. c. quantities of milk from each cow at brief intervals and later destroyed, subjected to the agglutination test for abortion disease, and cultured with the object of determining whether any of the cows had ceased to be carriers of the microorganism or were still producing milk infected by *Bact. abortus*.

On January 29, when guinea pigs Nos. 77497 and 77498 were destroyed and cultured, 43 guinea pigs previously inoculated with milk of the above-mentioned cows were also destroyed, autopsied, cultured, and subjected to the agglutination test for abortion disease. Cultures were made from the spleens of all the animals, using for medium the lot of serum-agar slants employed for culturing guinea pigs No. 77497 and 77498. Enough slants were inoculated with the spleen of each guinea pig to allow the incubation of a portion of the cultures under both CO_2 and normal atmospheric conditions. The suspected *Bacterium melitensis* cultures were placed in the same jars containing 10 per cent CO_2 as the *Bact. abortus* cultures. In other words, cultural conditions were identical.

Examination of the cultures incubated under normal atmospheric conditions on January 30 and January 31 failed to reveal any evidence of growth. On February 1 the tubes subjected to both methods of incubation were examined. Serum-agar slants from spleen of guinea pig No. 77497 showed small opalescent colonies under both methods of cultivation. Likewise serum-agar slants from testicle and spleen of guinea pig No. 77498 showed small colonies under both conditions. There appeared to be little or no difference in size of colonies under both methods of incubation.

Thirty-five serum-agar slants from spleens of the abortion experiment guinea pigs incubated in closed jars with 10 per cent CO_2 showed opalescent colonies indicative of *Bacterium abortus*. The colonies were considerably larger than those suspected of being *Bact. melitensis*. Under normal atmospheric conditions a like number of tubes showed no evidence of growth, although sown with spleen tissue of these same guinea pigs. The cultures were further subjected to the same conditions of incubation, and on February 5 the colonies developing from inoculations of guinea pigs Nos. 77497 and 77498 on serum-agar slants had increased considerably in size. The tubes under the two methods of incubation differed but slightly, if at all. Colonies from the inoculations of these guinea pigs were visible on 3 per cent glycerin-agar slants, and the infusion-bouillon cultures plainly showed evidence of rather luxuriant growth. The colonies in the closed jars developing from the abortion experiment guinea pigs appeared considerably larger than those from the *Bact. melitensis* guinea pigs. Under normal atmospheric conditions the serum-agar slants from the abortion experiment guinea pigs appeared sterile and remained so until February 12, when they were discarded.

While guinea pig No. 77497 showed well-marked lesions, the number of colonies developing from the spleen inoculations was less than 25 on any of the tubes of solid medium. Inoculations from the spleen of guinea pig No. 77498 resulted in the isolation of from 100 to 200 colonies each, although the lesions here were not pronounced.

Antigens prepared from the infections isolated from guinea pigs Nos. 77497 and 77498 were agglutinated by *Bacterium abortus* and *Bact. melitensis* antiserums in such a manner as to indicate that either *Bact. abortus* or *Bact. melitensis* was being dealt with.

Bacterium abortus was isolated from 20 of the 43 guinea pigs employed in the abortion experiment when the cultures were incubated in a partial CO_2 atmosphere. It is probable that numerous strains were represented, since in this test eight different cows used in the experiment were found to be still carrying the infection in their milk. Since numerous workers have observed that strains of *Bact. abortus* which have become accustomed to artificial media even after being injected into the bodies of bovines and multiplying for long periods can be readily cultivated again under normal atmospheric conditions, it may be well to state that no abortion vaccine had ever been used, nor artificial infection of any animals with *Bact. abortus* practiced in the herd where the abortion experiment was being conducted.

On February 14, when the remaining 15 guinea pigs, 14 of which had been inoculated with blood from the four goats, were destroyed, no lesions indicative of *Bacterium melitensis* infection were detected. Negative agglutination and negative cultural results for *Bact. melitensis* were obtained.

Since the isolation of the Texas strains of *Bacterium melitensis*, the writer has endeavored to determine with what frequency *Bact. abortus* may be isolated from the spleens of infected guinea pigs, following their inoculations with *Bact. abortus* infected milk, when sown on serum-agar slants in cotton-plugged tubes and placed in an incubator kept moist by the introduction of a receptacle containing water. Success has been attained only when the guinea pigs were inoculated with milk from cows which had been artificially infected with *Bact. abortus* strains accustomed to propagation in a normal atmosphere, although such tests have numbered between 75 and 100. Sealing the tubes after heating their upper portions caused isolation of the organism in some cases, but growth was slow as compared with the carbon-dioxid method.

THE WORK OF SOME PREVIOUS INVESTIGATORS

Bruce (*2*), the discoverer of *Bacterium melitensis*, in announcing his discovery makes no mention of using special medium or of incubating his cultures in other than the usual manner. Agar-agar nutrient jelly is recorded as having been used for medium. Some of his tubes sown with spleen tissue of a fatal human case were left at room temperature, and some subjected to 37° C. incubation. Under 37° incubation, growth was visible in 68 hours. At room temperature 168 hours elapsed before growth was observed.

Horrocks (*13*) describes the isolation of *Bacterium melitensis* from the milk of a goat by spreading milk sediment on litmus-nutrose-agar plates. After four days incubation at 37° C. colonies of the organism appeared on all the plates sown.

Shaw (*23*) describes investigational studies of 91 goats. The milk sediment of 30, which gave positive agglutination reactions, was sown on nutrose-litmus-agar plates. *Bacterium melitensis* was isolated from 7 of the number following incubation at 37° C. He further describes serological and cultural work in connection with 33 cows on the island of Malta. Ten of the number gave agglutination reactions varying from 1 to 30 to 1 to 1,000. From the milk sediment of 2 of this number *Bact. melitensis* is mentioned as having been repeatedly isolated, 3 plates yielding in some cases more than 200 *Bact. melitensis* colonies. The method described in the isolation of the organism consisted in centrifugalization of the milk samples and the sowing of the sediment on nutrose-litmus-agar in Petri dishes. Examination of the cultures was made after five days' incubation at 37°.

Keefer (*17*), in referring to the results of Shaw, suggested that Shaw was probably dealing with *Bacterium abortus*. The writer's experience has been that in the isolation of the abortion organism from the milk of naturally infected cows considerable difficulty is encountered in obtaining the original cultures, even when seemingly ideal conditions were provided. These observations and the results reported by different workers in this connection lead to the assumption that Shaw's conclusions may have been wholly correct, regardless of the fact that at the time the Malta fever investigations were made the close similarity in morphology, biochemical, and serological characteristics of the Malta fever and abortion organism was little suspected.

Huddleson (*15*), who deserves credit for greatly simplifying the method of isolation of *Bacterium abortus* from infected tissues and fluids, and the discovery that CO_2 gas in combination with air in the proper proportion is the essential factor rather than the degree of oxygen tension, states that to simplify the isolation of the organism from milk the following factors must be considered:

> The medium and its proper preparation; the proper H-ion concentration of the medium; the employment of an agent which will eliminate fast-growing organisms and the method of incubation.

Cooledge (*3*) in discussing the isolation of *Bacterium abortus* from milk of infected bovines calls attention to the defects of the Nowak method, in which cultures of *Bacillus subtilis* are grown in closed jars with those sown with suspected *Bact. abortus* infected materials. While he mentions having succeeded in isolating *Bact. abortus* through the use of this method he refers to it as—

> too tedious a process for application to a considerable number of samples, requiring weeks to isolate and identify the cultures,

a feature which prompted him to investigate the value of the agglutination and complement-fixation tests in connection with milk whey as a means of detecting *Bact. abortus* udder-infected cows.

Giltner, Huddleson, and Tweed (*10*), writing on *Bacterium abortus* udder infection in bovines, refer to the cultural requirements of the organism in milk, and ascribe Huddleson's high degree of success in its primary cultivation from the milk to the probability that a 10 per cent CO_2 atmosphere, which he utilized, exists within the udder, since, according to their statement—

> freshly drawn milk from the healthy udder shows approximately this pressure of CO_2.

Meyer and Shaw (*20*), on the contrary, seem of the opinion that the peculiar requirements of the abortion organism are observed only from uterine isolations, and refer to the results of McFadyean and Stockman (*19*), Holth (*12*), Schroeder and Cotton (*22*), Evans (*5*), and Steck (*24*) to substantiate their view.

Huddleson (*14*) has shown that sealed tubes slowly develop a 10 per cent CO_2 tension, the colonies developing when this is reached. Cooledge (*3*) refers to the claims of certain investigators that when tubes of agar are sealed and incubated there is a period when the proper amount of oxygen is present, owing to a partial absorption of oxygen by the agar. Some of the confusion regarding this feature may perhaps be ascribed to the fact that in the earlier investigations the writers may have considered certain details in the technic too inconsequential to mention, or that in the studies the investigators failed to take into consideration whether they were dealing with laboratory strains or with those which had never become accustomed to growth on artificial media. The writer was recently informed by Dr. W. E. Cotton that in the early isolations of the organisms from pigs infected with market milk, recorded by Schroeder and Cotton (*22*), sealing of the tubes was practiced, and that development of colonies required an incubation period of from a week to ten days.

Original cultures of *Bacterium abortus* obtained from swine appear to show different atmospheric requirements. Differentiation of porcine strains of *Bact. abortus* from *Bact. melitensis* by their cultural or atmospheric requirements may be difficult or even impossible. Good and Smith (*11*), in describing their success in isolating *Bact. abortus* from a case of swine abortion by the Nowak method, mention having observed the development of colonies on the fourth day. "The first subcultures," they state, "grew readily in the air, whereas subcultures derived from the cow usually do not grow in the air until after being cultured for several generations by the Nowak method." Since none of the original cultures were incubated in the air, they were unable to state whether growth would have taken place.

Doyle and Spray (*4*) find results seeming to indicate that the atmospheric requirements of the porcine organism may differ from those of *Bacterium abortus* (bovine), since they mention having isolated the organism after three days' incubation on serum-agar slants from guinea pigs inoculated with an emulsion of the placenta of a naturally infected sow. No mention is made of having subjected the cultures to other than normal atmospheric conditions.

SUMMARY

The isolation of *Bacterium melitensis* from guinea pigs infected by inoculations with blood from human cases of Malta fever, or with milk from goats affected with the disease, may be readily accomplished when serum-agar slants are sown with the infected tissues and incubated under normal atmospheric conditions at 37° C.

The original cultures develop with equal if not greater rapidity in a normal atmosphere than in one partially displaced by CO_2 gas. This characteristic permits the differentiation of *Bacterium melitensis* and *Bact. abortus* (bovine) in primary isolations.

Since artificially cultivated strains of *Bacterium abortus* do not promptly become exacting in their atmospheric requirements when again introduced into animals, the biological characteristics of such strains may be identical with those of *Bact. melitensis*.

The results of different investigators would suggest that the differentiation of *Bacterium melitensis* from porcine strains of *Bacterium abortus* may also be impossible by observations of their biological characteristics.

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FEED COST OF MILK PRODUCTION AS AFFECTED BY THE PERCENTAGE FAT CONTENT OF THE MILK[1]

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This paper is an elaboration of a subject which was touched incidentally by Gaines and Davidson (*3, p. 596*)[2] in a study of the relation between percentage fat content and yield of milk. In the last analysis feed cost in this paper refers to financial cost. Approach is made, however, through certain physiological principles and relationships which permit deductions as to feed cost in terms of nutrients.[3] From feed cost in terms of nutrients to relative feed cost in terms of dollars is a comparatively simple and direct step in the present case.

From a nutritional standpoint the feed cost of producing milk falls in two categories: (1) Maintenance requirements, that is, the nutrients required by the cow as not lactating; (2) lactation requirements, that is, the *additional* nutrients required as lactating. The term "maintenance" as here used includes the requirements for all metabolic activities except those associated with lactation. Such usage is common in our present-day feeding standards for cows in milk.

According to our feeding standards the nutrients required for maintenance and for lactation are approximately equal in the case of an 1,100-pound cow producing 9,277 pounds of 4 per cent milk per year. For a lower yield the maintenance requirement is the larger and for a higher yield the lactation requirement is the larger. Roughly speaking, in very good dairy practice the two categories of feed cost—maintenance and lactation—are of equal weight. Together they constitute the feed cost of milk production, and the problem is to express them as a function of the percentage fat content of the milk. We may consider, first, the lactation requirements; second, the maintenance requirements; third, the milk-production requirements; that is, the sum of the lactation and maintenance requirements; and fourth, the translation of this sum into terms of dollars.

THE LACTATION REQUIREMENTS

In the development of feeding standards the lactation and maintenance requirements were early distinguished. Haecker carried the development further by showing experimentally that the lactation requirements are dependent on the composition of the milk as well as on the amount of milk. Haecker (*4, p. 50*) made the fundamental observation " * * * that there was a remarkable uniformity in the net nutriment required for the production of a unit of milk solids when nutrients consumed and milk solids produced were reduced to carbohydrate equivalent." He accordingly formulated his feeding standard on the basis of the composition of milks of varying fat percentage as indicated by analyses made in his own laboratory. His formulation on this basis has stood the test of several years of practical use and the scrutiny of considerable experimental investigation.

Haecker's observation quoted above is a key for attack of the lactation requirements. His "carbohydrate equivalent" of the milk solids is equivalent to energy value of the milk solids. And the energy value of milk solids in a given amount of milk may be simply expressed as a function of the fat percentage of the milk. If we take one pound as our unit amount of milk, and measure energy value in large calories, letting E represent energy value, and, t, percentage fat content of the milk we have,[4] $E=49.64\ (2.66+t)$. If Haecker's generalization is correct the nutrients required for lactation are directly proportional to $49.64\ (2.66+t)$, and may be expressed as $K_L\ (2.66+t)$ where K_L is a constant. His published data permit of detailed examination as to how well this generalization is supported by his experimental observations.

Haecker's published data pertain to 142 individual feeding trials with

[1] Received for publication April 1, 1924—issued March, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 601.

[3] The term "nutrients" as used throughout this paper refers to total digestible nutrients of the feed, digestible fat being multiplied by the factor 2.25.

[4] For the derivation of this equation see Gaines and Davidson (*3, p. 562*).

an average length of 148 days, and include for each trial, and for each cow, expressed as a daily average: (1) Milk yield; (2) per cent fat (3) nutriment daily; (4) nutriment for maintenance; and (5) nutriment for product. The "nutriment daily" (corresponding to milk production requirements as used here) was estimated from the weights and analyses of the feeds consumed by the cow. The "nutriment for maintenance" was estimated from the live weight of the cow, allowing 7.92 pounds of nutrients per day per 1,000 pounds live weight (*4, p. 10*). The "nutriment for product" (corresponding to lactation requirements as used here) was estimated as "nutriment daily" minus "nutriment for maintenance." Haecker's data may be reworked and rearranged to better serve the present purpose. From his data the writer has calculated the lactation requirements *per pound of milk* and quite diverse also in breeding—Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, Shorthorn, crossbred, and native.

The mean nutrients requirement for lactation per pound of milk for each of the fat percentage classes has been derived from Table I and the data are shown graphically in Figure 1. It is evident at once from the figure that the nutrients for lactation may be expressed as a linear function of the fat percentage. We have now to determine a value for K_L, in a curve of the of the equation $N_L=K_L\ (2.66+t)$, for these particular data and see how its slope conforms to the observed results. The curve is simply fitted so that the algebraic sum of the deviations is zero, and this gives a value of 0.049 for K_L. The calculated values are given in Table II and graphically in Figure 1. The agreement with the observed values is very close indeed.

TABLE I.—*Correlation of the variables, percentage fat content of milk and nutrients for lactation per pound of milk* [a]

Pounds of nutrients per pound of milk-class mid-points	Percentage fat content of milk—class mid-points								
	2.55	3.05	3.55	4.05	4.55	5.05	5.55	6.05	Total
0.215	1	3	1	1					6
.245	1		2	1					4
.275	2	3	7	1	6	1	1		21
.305		6	3	3	6	3			21
.335		1	6	2	9	2			20
.365		1	2	3	7	6	4		23
.395	[b] 1			1	1	11	7		20
.425	[b] 1		2	2	1	3	5	2	15
.455					1	1	4	1	7
.485							2		2
.515						1			1
Total	4	14	23	14	31	28	23	3	140

$r=0.6529\pm0.0327$

[a] Data from Minn. Bul. No. 140.

[b] These two records are from the same cow (Lou II), stated in the text (*p. 20*) to have been out of condition at the time. It has seemed advisable to exclude these records in the computations.

correlated this result with the per cent of fat in the milk. Table I shows the correlation surface.

The coefficient of correlation, 0.6529 $\pm$0.0327, is perhaps not so high as one might expect to find in a relation of this kind. It must be borne in mind, however, that there are several other variables involved here, such as variability in the estimated consumption of nutrients as compared with the actual (including loss of body tissue); and the estimated maintenance requirements as compared with the actual (including gain of body tissue). The 46 cows used in Haecker's experiments ranged in age from 2 to 14 years, and in weight from 593 to 1,347 pounds. They were

TABLE II.—*Lactation requirements per pound of milk as affected by percentage fat content of milk* [a]

Per cent of fat in milk (t)	Number of observations	Nutrients per pound of milk	
		Observed values	Calculated values, N_L [0.049 (2.66 +t)]
2.55	4	0.253	0.255
3.05	14	.286	.280
3.55	23	.310	.304
4.05	14	.333	.329
4.55	31	.333	.353
5.05	28	.380	.378
5.55	23	.409	.402
6.05	3	.435	.427

[a] Data from Minn. Bulletin 140.

Haecker's (*4*) standard and Morrison's (*5*) standard (mean of his range values) are included in the graph. It will be observed that Haecker's standard does not correspond as closely to his own observations as does Morrison's standard. Morrison's standard presumably represents his summary of all the available evidence on the relation.

The relation between the composition of the milk and the nutrients required for lactation, under comparable conditions of feeding, may be stated thus: *The nutrients required for lactation are directly proportional to the energy value of the milk solids.* This relation should be known as HAECKER'S LAW.

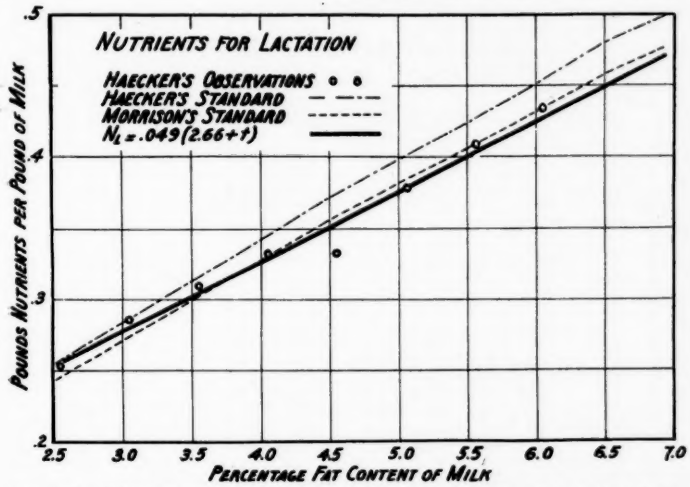

FIG. 1.—Lactation requirements per pound of milk as affected by percentage fat content of milk.

In the equation, N_L=0.049 (2.66+t), to the solid line curve, N_L=pounds of nutrients per pound of milk, and t=per cent of fat in the milk.

Coming back to the present viewpoint, it may be concluded, at least so far as commercial milk production is concerned, that the lactation requirements, N_L, may be expressed as a function of the fat percentage of the milk, t, by the equation:

$$N_L = K_L\ (2.66+t)$$

in which K_L is a constant *so far as affected by fat percentage.*

THE MAINTENANCE REQUIREMENTS

We have next to consider the maintenance requirements per unit of milk in relation to the percentage fat content of the milk. Haecker based his maintenance estimate strictly upon the weight of the cow. This is common scientific practice of the present time and we may assume that it is justified in the case of the milk cow.

One of the first questions to answer is, is there any correlation between size (maintenance requirements) of the cow and percentage fat content of the milk? Eckles (*2, p. 403*) is authority for the statement that "there is no relation within the breed between the size of the animal and the richness of the milk." Statistical evidence on this point is summarized in Table III. This table gives the mathematical constants of the correlations between certain size measurements (largely weight) of the cow and the percentage fat content of the milk as derived from published records indicated in connection with the table.

From Table III it is plain that weight and fat percentage are entirely independent within any one of the breeds represented. Therefore (within the same breed) the maintenance requirements are independent of fat percentage, and, *so far as affected by fat percentage*, the maintenance requirements are a constant, K_m. The maintenance requirements per pound of milk are, therefore,

$$\frac{K_m}{\text{milk yield in pounds}}$$

TABLE III.—*Mathematical constants of the correlations between size of cow and percentage fat content of milk* [a]

Breed and source of records [b]	Age of cow	Number of records	Size of cow, pounds or inches				Fat percentage			Coefficient of correlation
			Measurement	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation	
	Years									
Jersey R. M.	1	623	Weight	741.2± 2.5	90.7 ±1.7	12.2±0.24	5.448±0.015	0.568±0.011	10.4±0.20	0.008±0.027
Do.	2	2,308	do.	795.4± 1.3	92.6 ± .9	11.6± .12	5.457± .008	.546± .005	10.0± .10	− .037± .014
Do.	3	1,348	do.	857.6± 1.7	91.6 ±1.2	10.7± .14	5.438± .010	.531± .007	9.8± .13	− .001± .018
Do.	4	1,068	do.	899.5± 2.1	99.9 ±1.5	9.4± .14	5.427± .011	.527± .008	9.7± .14	.040± .021
Do.	5	905	do.	921.3± 2.2	95.9 ±1.5	10.4± .17	5.368± .011	.500± .008	9.3± .15	− .008± .022
Do.	6	641	do.	930.6± 2.6	99.2 ±1.9	10.7± .20	5.351± .015	.554± .010	10.4± .20	− .009± .027
Do.	7	486	do.	931.4± 3.0	98.5 ±2.1	10.6± .23	5.360± .016	.553± .012	9.9± .21	.019± .031
Do.	8+	725	do.	939.2± 2.4	94.5 ±1.7	10.1± .18	5.282± .013	.517± .009	9.8± .17	− .012± .025
Do.	All ages	8,104	do.	861.1± .9	114.6 ± .6	13.3± .07	5.409± .004	.538± .003	9.9± .05	− .041± .007
Guernsey W. C. C.	do.	124	do.	979 ± 7.4	122.5 ±5.2	12.5± .54	5.080± .028	.467± .020	9.2± .39	.010± .060
Holstein W. C. C.	do.	145	do.	1,234.6±10.3	184.4 ±7.3	14.9± .60	3.444± .016	.284± .011	8.2± .32	.001± .056
Holstein A. R. O	do.	416	do.	1,098.5± 5.5	167.6 ±3.9	15.2± .36	3.510± .012	.376± .009	10.7± .25	.088± .033
Do.	do.	416	Height	51.9± .07	2.20± .05	4.2± .10	3.510± .012	.376± .009	10.7± .25	.028± .033
Do.	do.	416	Length	61.8± .13	4.03± .09	6.5± .15	3.510± .012	.376± .009	10.7± .25	.067± .033

[a] The class intervals used in making up the correlation surfaces from which the data of this table are derived were as follows: For fat percentage, 0.1; for size, 50 pounds in weight, 1 inch in height at withers, and 2 inches in body length. The weights are for the most part estimates and there is a tendency to estimate at even 100 pounds; a lesser tendency, at 50 pounds, and occasionally, at 25 or 75 pounds. As an offset to this tendency the class ranges were chosen as 376–424, 425–475, 476–524, 525–575, etc. A similar plan was followed in the linear measurements to offset a tendency to even inches, to a lesser extent one-half inches, and occasionally one-quarter or three-quarters inches.

[b] The Jersey records are from the Register of Merit, vols. 1916, 1917, 1918, and 1919, all long-time records except R. M. No. 6394 (estimated weight 1950 pounds); the Guernsey and Holstein Wisconsin Cow Competition records are from the Wisconsin Cow Competition, Wisconsin Res. Bul. No 26; (long-time record); the Holstein Advanced Register O records are from the Advanced Register, vols. 12–17 inc., all 7-day records giving weight, shoulder height, and body length of cow.

The next question is as to the relation between fat percentage and milk yield. Gaines and Davidson (*3*) have shown that in so far as yield is affected by percentage fat content, the milk yield is inversely proportional to the energy value of the milk solids per unit milk and may be expressed as: $\frac{a}{2.66+t}$, in which a is a constant, affected in value only by factors other than fat percentage. The maintenance requirements per pound of milk, N_M, are therefore

$$\left(\frac{K_m}{\frac{a}{2.66+t}}\right)=\frac{K_m\,(2.66+t)}{a}$$

and

$$N_M=K_M\,(2.66+t)$$

where K_M is a constant $\left(\frac{K_m}{a}\right)$ *so far as affected by fat percentage.*

Since size is independent of fat percentage, it follows that growth is likewise independent of fat percentage; hence the inclusion of growth requirements in the maintenance requirements involves no error from the present standpoint. The same reasoning may be applied to fœtal growth. Obviously, however, when we include different breeds there may be some correlation (presumably negative) between fat percentage and size. If the lower testing cow is a larger cow this will imply increased maintenance and increased yield, both of which are factors in the maintenance requirements per pound of milk. The point is further considered below.

THE MILK PRODUCTION REQUIREMENTS

The total nutrients, N_T, required for milk production are the sum of the lactation and maintenance requirements. That is, $N_T=K_L\,(2.66+t)+K_M\,(2.66+t)=K_T\,(2.66+t)$.

As an intrabreed relation, then, the nutrients required for milk production, *so far as affected by fat percentage,* are K_T, $(2.66+t)$ where K_T is a constant (K_L+K_M). (The value of K_T is, of course, greatly affected by factors other than fat percentage.)

As an interbreed proposition there may be a relation between size and fat percentage such as to result in a difference in the value of the constant K_T for the different breeds. An increase in maintenance requirements tends to increase the value of K_M and an increase in production tends to decrease the value of K_M. Since the maintenance requirements vary directly as the weight of the cow, it follows that the value of K_M, and hence also the value of K_T, will vary unless production [milk yield $\times$ $(2.66+t)$] varies also directly as the weight of the cow. The relation between size of cow and production, as between different breeds, has not been adequately determined.[5] As a practical way of considering the matter, however, we may calculate the values of K_T for Holstein cows and Jersey cows, as representing the extremes in size and percentage fat content of milk among our common dairy breeds, and note how the values of K_T for these two breeds compare.

Table IV gives the values of K_T calculated on the basis of Haecker's quantitative relations as,

$$K_T=\frac{0.049(2.66+t)\text{milk yield}+(0.00792\times 365\times\text{weight})}{\text{milk yield}\,(2.66+t)}$$

[5] Various studies of the relation between size of cow and yield indicate clearly that production varies with size. Brody, Ragsdale, and Turner (*1*), from the yearly official test records of Jersey cows found that the relation between size and fat yield is expressed by the general equation, $F=aW+b$, in which F is fat yield, in pounds, W is weight of cow, in pounds, and a and b are constants. Accordingly, the nutrients for maintenance per pound of fat yielded are $\frac{2.89\,W}{aW+b}$ and increase with increasing value of W; that is, the smaller cow is more efficient than the larger. Where age is held constant a has a value near 0.2 and b near 250, and the variation in nutrients for maintenance per pound of fat, with variations in weight of the cow, is of considerable magnitude. When cows of ages 2 to 9 years are included the following equation, derived from their Table II and using their notation, describes the relation: $F=0.373\,W+104$.

Pearson (unpublished data, Illinois Experiment Station) in a study of the yearly records of 642 grade Holstein cows, unselected as to age, found a positive correlation between weight and milk yield, $r=0.304\pm0.024$. The regression line is linear, and the mean milk yields of the several weight classes are closely expressed as 6.15 times the weight. The mean fat percentage was 3.42 ± 0.01 and since fat percentage is independent of weight, Pearson's data may be translated in terms of fat, using the above notation as: $F=0.210\,W$. From the present standpoint this equation differs fundamentally from the one above for Jersey cows. Whether the difference is a matter of breed (heredity) or environment can not be said.

TABLE IV.—*Calculated values of K_T for Holsteins and Jerseys from cow-testing association and advanced registry records*

Records	Number of records	Milk	Fat	Weight	Pounds nutrients for—			K_T
					Lactation	Maintenance	Total	
		Pounds	*Per cent*	*Pounds*				
Holstein C. T. A	2,773	7,218	3.54	1,038	2,193	3,001	5,194	0.116
Jersey C. T. A	970	5,364	5.14	925	2,050	2,674	4,724	.113
Holstein A. R	5,266	14,887	3.41	1,150	4,428	3,324	7,752	.086
Jersey R. M	8,038	8,005	5.41	861	3,165	2,489	5,654	.087

The milk yields and fat percentages are from data by Gaines and Davidson (*3, p. 619*); the weights for the advanced registry records are from Table III; the weight for the Holstein C. T. A. records is the average of 793 cows included in the total (2,773); and the weight for the Jersey C. T. A. records is an estimate based on personal observation of the cows concerned.

It will be noted from Table IV that the values of K_T for Holstein and Jersey cows from the cow-testing association records are very nearly the same, 0.116 and 0.113, respectively. The value of the constant, K_T, corresponds to the theoretical pounds of nutrients required per unit of energy value (49.64 large calories) of the milk solids. K_T is virtually an inverse index of cow efficiency. The breeding and conditions of management of the cows concerned here are quite comparable. A large proportion (about 90 per cent) of the cows were high grades and the balance purebreds. The feeding and management were those of good commercial practice in the whole-milk districts of Illinois. It is quite probable, as will be shown later, that the calculated value of K_T under these conditions is very close to the actual value.

Compared with the records of the cow-testing association, the advanced registry records show a lower calculated value for K_T, namely, 0.086 for the Holstein and 0.087 for the Jerseys. But, again, under the conditions of official testing, the values for the two breeds are practically equal. The lower value of K_T is due to the higher level of production. Since many of the records included here are the result of deferred breeding and continuous milking throughout the year as well as extravagant feeding, the indicated values of K_T do not represent practical commercial milk production.

The point of immediate interest, however, is whether the relation $N_T = K_T\ (2.66+t)$ holds as between breeds. While the evidence is not as precise as may be desired, it does indicate so far as it goes that the relation holds reasonably close between breeds.

FEED COST IN DOLLARS

Feed cost in nutrients may be translated into feed cost in dollars by determining the price of nutrients in the feeds used. There is very little difference in the character of the ration required for milk production so far as affected by fat percentage of the milk. The lower-testing cow requires a slightly greater proportion of protein but that the effect of this difference on cost per pound of nutrients is quite negligible may be inferred from data of Ross, Hall, and Rhode (*7*). They show the cost per pound of nutrients (pasture not included) to be nearly constant so far as affected by yearly fat yield of the cow. Thus, as the yield of fat increased from 161 to 361 pounds, the cost per pound of nutrients in the feed consumed increased by less than 3 per cent, as an average of the five years 1908 to 1912.

From this it seems safe to assume that the cost per pound of nutrients is practically unaffected by the fat percentage of the milk. Hence, feed cost in dollars is proportional to feed cost in nutrients, and *so far as affected by percentage fat content of the milk*

$$FC = K\ (2.66+t)$$

in which FC is feed cost ($) per pound of milk; t is per cent of fat in the milk; and K is a constant dependent in value on the price level of feeds and on the value of K_T.

While the point under consideration here is *relative* costs, rather than *absolute* costs, it is perhaps worth while to draw a comparison as to absolute feed cost. Pearson (*6*), by cost accounting methods, found the cow feed cost of milk production to be $1.05 per hundredweight of milk for

the years 1914–1916, in the Illinois Chicago district. The average fat test was 3.56 per cent. The conditions of production were quite similar and entirely comparable to the conditions of the Holstein cow testing associations referred to in Table IV. Using the data of Ross for 1914–1916 the average cost of digestible nutrients was $0.0153 per pound. This figure may be taken to represent the price level of feeds at the time of Pearson's investigation and on this basis $K = \$0.0153\ K_T$, or, taking K_T from Table IV, $K = \$0.0153 \times 0.116 = \$0.00177$ Substituting these values in the feed cost equation we have $FC = \$0.00177\ (2.66 + 3.56) = \$0.011$. That is, the feed cost calculated by the equation is $1.10 per hundredweight, corresponding quite closely to Pearson's cost-accounting data, $1.05 per hundredweight. Pearson's silage evaluation is lower than that used by Ross. There is also the question as to the cost of nutrients from pasture in Pearson's data (pasture is not included in Ross's data). On the whole, the feed-cost equation above may be regarded as in accord with Pearson's cost-accounting data.

COSTS OTHER THAN FEED

The data on feed costs as interpreted above are, to the writer's mind, quite conclusive. The costs other than feed constitute a separate problem to be solved by cost-accounting methods rather than by biological methods.[6] Until the economists help us out in the matter it may be assumed tentatively without any apparent gross error that the other costs are roughly proportional to feed costs. This assumption does not imply that there is a constant ratio of feed cost to total cost, but merely that under conditions where, for example, feed cost is 60 per cent of the total cost of milk production for the 3 per cent cow it will be 60 per cent also for the 4 per cent cow under the same conditions. As between breeds, especially, there are a number of cost factors directly or indirectly involved, such as cost of milk hauling, beef value of cow, veal value of calves, etc., but these are all minor items. If we may assume that the total cost of milk production is proportional to feed cost, *so far as affected by fat percentage*, it is possible to make some comparisons with reference to the price differential for fat percentage in whole-milk markets.

THE DIFFERENTIAL IN WHOLE-MILK PRICES

The great bulk of the whole-milk market is for city supply. Judging by the August, 1923, "Fluid Milk Market Report for the United States" of the United States Department of Agriculture,[7] the markets of about 11 per cent of our cities still retain the flat-rate basis of payment, while 89 per cent make some sort of distinction or differential in price according to the fat test of the milk. Both the producer and the buyer are interested in this connection in the effect of fat percentage on cost of production. The buyer's price is an expression of demand, and the producer seeks to adjust his production to that demand, as expressed in price, which offers the largest profit. If the buyer wishes milk of a certain fat test he will discriminate in price against milk of another fat test out of proportion to the cost of production. On the other hand, if he wishes to encourage equally the production of milk of various fat tests he will pay in proportion to the cost of production, as affected by percentage of fat content.

According to the equation developed above, the cost of production, *so far as affected by fat percentage*, may be expressed as a function of the fat percentage, and likewise the difference in cost of production, corrresponding to a certain difference in fat percentage, may be expressed as a function of the cost of production of milk of any definite or base fat percentage. If we take 3.5 as the base fat percentage and a "point" or 0.1 as the difference in fat percentage, then the difference in cost per point is $\left(\frac{0.1}{2.66+3.5}\right) C_{3.5}$, where $C_{3.5}$ is the cost of 3.5 per cent milk. That is, if the price differential is proportional to the cost differential it will be 1.6234 per cent of the price of 3.5 per cent milk. In like manner, it will be 1.7668 per cent of the price of 3 per cent milk; 1.5015 per cent of 4 per cent milk; or 1.3055 per cent of 5 per

[6] Some may regard the whole matter as a problem for the cost accountant alone. One can scarcely doubt, however, that the effect of fat percentage on cost is a material factor in the dairyman's profit, and has been especially so in the past when the flat rate for whole milk was so generally in effect. But for some reason the economist seems to have passed by this item in his rather detailed analyses of farm management and profits.

[7] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF AGRICULTURAL ECONOMICS, MARKET NEWS SERVICE. FLUID MILK MARKET REPORT FOR THE UNITED STATES, AUGUST, 1923. 4 p. 1923 [Mimeographed].

cent milk. A price differential greater than that indicated is favorable to the production of high-testing milk; while a smaller differential is favorable to the production of low-testing milk.

Obviously, the price differential should be considered relative to the price of milk. Figure 2 shows in the form of a frequency distribution curve the differentials for 101 cities taken from the August, 1923, market report above referred to.

The differential is expressed as a per cent of the price[8] of 3.5 per cent milk, and all cities are included where a definite price and differential are given. For the sake of comparison the cost of production differential is indicated in the figure, expressed likewise as a per cent of the cost of 3.5 per cent milk. Except for the two classes, 0 and 2.8, there appears to be a tendency toward a normal distribution curve centering about the cost of production as a mean. The class at 0 represents those cities paying a flat rate. The class at 2.8 represents those cities paying, practically, on a straight fat basis (a differntial of 2.857 per cent of the price of 3.5 per cent milk is a straight fat basis).

An exact adjustment of price in proportion to cost is rather cumbersome by the system of a certain allowance per 0.1 per cent in the fat test. It may be more simply accomplished by adjusting the price according to one of the following formulæ, in which t stands for fat percentage and $P_{3.0}$, the price of 3.0 per cent milk; $P_{3.5}$, the price of 3.5 per cent milk; etc.:

$$0.17668\ P_{3.0}\ (2.66+t)$$
$$.16234\ P_{3.5}\ (2.66+t)$$
$$.15015\ P_{4.0}\ (2.66+t)$$
$$.13055\ P_{5.0}\ (2.66+t)$$

or, generalized,

$$\left(\frac{1}{2.66+b}\right)(P_b)\ (2.66+t)$$

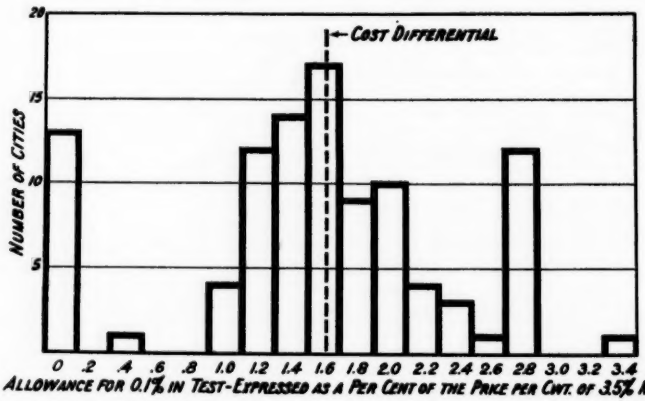
← Cost Differential
Number of Cities
20
15
10
5
0 .2 .4 .6 .8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4
Allowance for 0.1% in Test-Expressed as a Per Cent of the Price per Cwt. of 3.5% Milk

FIG. 2.—Frequency distribution curve of 101 cities with respect to the differential in the price of milk according to the fat test of the milk. Data from August, 1923, "Milk Market Report." The differential is the price difference per hundredweight of milk for each difference of 0.1 in the per cent of fat in the milk. In order to make the data comparable it is expressed, in this figure, as a per cent of the price per hundredweight of 3.5 per cent milk. The broken line at 1.6 indicates the cost of production differential; that is, the difference in cost of production per hundredweight of milk for each difference of 0.1 in the per cent of fat in the milk (so far as the cost of milk production is affected by percentage fat content of the milk). The cost differential is expressed, likewise, as a per cent of the cost of production of 3.5 per cent milk. It is assumed that the cost of production is proportional to the feed cost

To illustrate the working of the above scheme, we may take the Chicago market for August, 1923, \$3.20 per hundredweight for 3.5 per cent milk. The price for milks of other test, to bear the same ratio to cost of production, would be \$0.16234 × 3.20 $(2.66+t)$ and \$0.52 $(2.66+t)$ would be the general expression for the price of milk for that month. Thus, 3.21 per cent milk would be \$0.52 $(2.66+3.21)$ =\$3.05; 4.14 per cent milk would be \$0.52 $(2.66+4.14)$=\$3.54; etc. The prices actually paid were \$3.08 and \$3.46, respectively.

SUMMARY

The nutrients required for lactation per pound of milk, N_L, may be expressed as a function of the fat per-

[8] This price is f. o. b. city. It would be better for this comparison to use country buying prices and weighted average for the year. The data for such a comparison are not available to the writer.

centage of the milk, t, as: $N_L=K_L(2.66+t)$. The nutrients required for maintenance per pound of milk, N_M, (because of the nature of the relation existing between fat percentage and size of cow and between fat percentage and milk yield) may also be expressed as a function of fat percentage, as: $N_M=K_M(2.66+t)$. Consequently the total nutrients required for milk production per pound of milk, N_T, may be expressed as: $N_T=K_T(2.66+t)$. The cost in dollars per pound of nutrients is practically unaffected by fat percentage and consequently the feed cost ($) per pound of milk, FC, may be expressed, as: $FC=K(2.66+t)$. In these relations K_L, K_M, K_T, and K are constants *so far as affected by fat percentage.*

The price differential for fat test as judged by the whole-milk markets of 101 cities seems to be tending to distribute about a mean which is approximately the same as the cost of production differential. The price of milk may be adjusted in proportion to the cost of production by the equation $P=\frac{P_b}{2.66+b}(2.66+t)$, in which P_b is the base price, b is the fat percentage to which the base price applies, and P is the price to be paid according to the fat test, t, of the milk delivered. This assumes that the cost of production is proportional to the feed cost of milk production, *so far as affected by fat percentage.*

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RELATION BETWEEN THE DIET, THE COMPOSITION OF THE BLOOD, AND THE SECRETION OF MILK OF DAIRY COWS [1]

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INTRODUCTION

It has been shown that with milking cows the lactose, fat, and proteins of milk are made in the mammary gland from glucose (*10*),[2] phosphatide (*11*), and amino acids (*3*), respectively, which are taken directly from the plasma of the blood. Meigs (*12*) has pointed out that within the limits of the experimental errors involved the mammary gland takes these precursors out of the plasma in approximately the proportion in which the corresponding constituents occur in the milk. This, as he points out, furnishes strong evidence for the view that the lactose, fat, and proteins of milk are, respectively, derived entirely from the dextrose, phosphatide, and free amino acids of the blood.

Abrupt changes in the rations of milking cows frequently produce decided changes in the yield and composition of milk. The concentration of some of the milk precursors in the blood plasma may be determined with a fair degree of accuracy. A study of the composition of the rations, blood, and milk simultaneously might, therefore, be expected to throw much light on important problems in the physiology of milk secretion. It would also throw light upon the relation between the diet and the composition of the blood with animals where the process of absorption on a given ration is practically constant. The cow furnishes an excellent subject in many respects for such a study.

The plasma amino N in milking cows is low (*2*, *3*). This is especially true when they are fed according to the ordinary standards of feeding. It is seldom so high as 3 mg. per 100 c. c. On an average, 25 per cent of this is taken out in a single passage of the blood through the gland. This means a very great depletion of some of the individual amino acids. It would seem likely, a priori, that the yield and composition of milk might, therefore, frequently be affected and limited by changes in either the concentration or composition of this amino-acid mixture. Hence, especial attention in this study has been given to the relation of the amino acids of the blood to the diet and the secretion of milk.

In the experiments to be reported milking cows were used. In their rations the protein was varied in amount or quality or both, and the energy content was varied either alone or together with the amount of protein. The amino N of the blood and plasma was determined at intervals. In some experiments it was found possible to follow simultaneously the amino N and the concentration of a particular amino acid (tryptophane) in the blood and thus to get some idea of the qualitative changes occurring in the blood mixture of amino acids. The glucose and lipoid P of the blood and plasma were determined in some experiments. The yield of milk and its content of N and fat were followed throughout. The milk lactose was determined in some experiments.

EXPERIMENTAL ROUTINE

The experiments, with the exception of No. VI, were begun with cows on rations which were adequate, or nearly adequate, in both protein and energy, according to the commonly accepted feeding standards for maintenance and milk yield (*7*, *p. 133*). The initial periods on approximately adequate rations were followed by periods in which the rations were made markedly inadequate in either energy or protein content, or both, the protein mixture in the ration being changed in either quantity or composition, or both. These periods of inadequate feeding were generally followed by periods in which the adequacy of the rations was again restored. The changes in rations were made abruptly and without transition periods.

Except for the hay, the feed was given in two equal portions, one between 4 and 5 a. m. and the other between 1 and 2 p. m. The daily allowance of hay was fed at the afternoon feeding. Each cow was milked at the same time daily (4.30 to 4.45

[1] Received for publication May 12, 1924—issued March, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 624.

a. m. and at 3.15 to 3.45 p. m.); and, so far as possible, the milking was done by the same man throughout each experiment. The blood samples were taken at 8.30 to 9 a. m.

In all the experiments except Nos. I and II the Ca, P, and Na contents of the rations were kept constant by adding varying amounts of ground limestone, $Na_2HPO_4.12H_2O$, and NaCl, as shown in Table II. The calculations of the amounts of these salts to be added were based on determinations found in the literature or made at various times in this laboratory. The cows also had access to NaCl when turned out daily in a bare lot.

ANALYTICAL METHODS

The samples of blood in Experiments I, II, III, V, and VIII were 450 to 1,000 c. c. They were collected in bottles containing 0.1 gm. of sodium oxalate per 100 c. c. of sample. In Experiments IV, VI, and VII the samples of blood were larger; and 0.7 c. c. of a solution of sodium citrate (38 gm. per 100 c. c. of solution) was used per 100 c. c. blood. The plasma was immediately separated by centrifuging 20 minutes at 3,600 revolutions per minute. The analytical samples for the blood and plasma determinations were taken out as quickly as possible.

The lipoid phosphorus of the blood plasma was calculated as the difference between the total and the inorganic phosphorus in the plasma as determined according to the methods described by Meigs, Blatherwick, and Cary (*11*). The amino acid N of the blood and plasma was determined as previously described (*3*) except that, beginning with the sample of July 22, Experiment I, the urease was reduced to one-tenth that previously used. The determinations on the Van Slyke apparatus were made at the same room temperature throughout each experiment. The reducing sugar of the blood and plasma was determined by the revised method of Folin and Wu (*6*). The blood corpuscle volume was measured off on a marked centrifuge tube.

The p-dimethylamidobenzaldehyde reagent of Herzfeld (*8*) was used in the determination of the tryptophane of the blood and blood plasma. The protein was precipitated either as in the amino N determination (cow 246), or by coagulation with acetic acid as in the amino N determination and subsequent precipitation with aldehyde-free alcohol[3] (cow 423). The tryptophane in the concentrated protein-free extracts was precipitated in the presence of 7 per cent (by volume) of sulphuric acid and 20 per cent of mercuric sulphate. The mercury-tryptophane precipitate was redissolved in H_2O and 5 per cent NaCN (total volume=5 c. c.). Then 2 c. c. of the reagent and 23 c. c. of hydrochloric acid were added. With cow 246 hydrochloric acid that had been saturated at 0° C. was used. With cow 423 ordinary hydrochloric acid was used and then HCl gas was run in slowly for 10 minutes, while cooling in tap water. They were then heated at about 60° C. in a water bath until the color was fully developed (30 minutes). They were compared with tryptophane standards similarly treated with the Herzfeld reagent, HCl, etc. Generally, three or more standards were run, because, although they usually agreed well, it was found that sometimes they did not. This method of determining the protein-free tryptophane is not entirely satisfactory; the colors do not match well. In Experiments IV and VI the unknowns and standards were diluted 1:2 with water before reading. The writers believe that the data for free tryptophane obtained by them are sufficiently reliable to warrant publication and the conclusions that they have drawn from them. Further work is in progress upon methods of determining free tryptophane in blood and plasma. The results of these investigations will be published later.

The milk was preserved by adding to each 100 c. c. either 10 drops of chloroform (Experiment I), 2 c. c. of formalin (Experiments II, III, V, and VII), or 2 c. c. of a mixture of 50 gm. of phenol and 10 c. c. of 95 per cent alcohol (Experiments IV, VI, and VII). The nitrogen in the milk was determined by the Kjeldahl-Gunning-Arnold (*1*) method, the fat by the ordinary Babcock method, and the lactose by means of a polariscope. The proteins in the lactose determinations were precipitated by acid mercuric nitrate according to the "Methods of Analysis" of the Association of Official Agricultural Chemists (*1*).

The analytical data shown in the tables are generally averages of two or more determinations except where lactose was used, in which case only single determinations were made. The tryptophane determinations in Experiment VIII were not run in duplicate.

The changes made in the rations and the effects thereby brought about on the composition of the blood and milk and on the quantity of milk yielded are given in detail in Tables I to X.

[3] Refluxed and distilled off from KOH.

TABLE I.—*General schedule of experiments*

Period No.	Dates		Digestible feed [a] daily					Remarks
			Offered [b]			Feed offered, as percentage of feed required [c]		
	From—	To—	Protein	Nutrients (total)	Fat	Protein	Nutrients	
			Kg.	*Kg.*	*Kg.*			
EXPERIMENT I.—COW 17. ENERGY AND PROTEIN CONTENT OF RATION REDUCED								
1	May 1, 1920	May 11, 1920, a. m.	0.864	6.21	0.321	82.4	85.8	Nearly adequate ration.
2	May 11, 1920, p. m.	June 7, 1920, a. m.	.494	4.11	.201			Energy and protein reduced in amount, no change in quality of protein.
3	June 7, 1920, p. m.	June 24, 1920	.864	6.21	.321			Nearly adequate ration.
EXPERIMENT II.—COW 54. ENERGY OF RATION REDUCED								
1	Nov. 25, 1920	Nov. 27, 1920	1.037	7.34	0.231	98.2	95.8	Nearly adequate ration.
2	Nov. 28, 1920	Dec. 24, 1920	1.036	4.89	.159			Energy of ration reduced, no change in protein.
3	Dec. 25, 1920	Jan. 15, 1921	1.037	7.34	.231			Nearly adequate ration.
EXPERIMENT III.—COW 422. PROTEIN REDUCED IN QUANTITY, NO CHANGE IN QUALITY								
1	Sept. 16, 1921	Sept. 21, 1921	0.902	6.59	0.299	103.7	102.2	Nearly adequate ration.
2	Sept. 22, 1921	Oct. 10, 1921	.471	6.63	.222			Protein reduced in quantity, no change in quality.
3	Oct. 11, 1921	Oct. 29, 1921	.902	6.59	.299			Nearly adequate ration.
EXPERIMENT IV.—COW 246. PROTEIN REDUCED IN QUANTITY, NO CHANGE IN QUALITY								
1	Oct. 27, 1922	Nov. 4, 1922, a. m.	1.225	7.29	0.277	92.1	88.8	Nearly adequate ration.
2	Nov. 4, 1922, p. m.	Dec. 9, 1922	.620	7.25	.257			Portein reduced in quantity, no change in quality.

[a] The digestible constituents of the feed are calculated from tables given by Henry and Morrison (*7 Appendix, Table III*).

[b] Feed offered was completely consumed in Experiments I, II, IV, V, and VIII. The feed refused in Experiments III, VI, and VII is indicated in footnotes to Tables V, VIII, and IX, respectively.

[c] Requirements based on Savage standard for feeding of milking cows (*7 p. 155*).

TABLE I.—*General schedule of experiments*—Continued

EXPERIMENT V.—COW 54. PROTEIN REDUCED IN QUANTITY AND QUALITY

Period No.	Dates		Digestible feed daily					Remarks
	From—	To—	Offered			Feed offered, as percentage of feed required		
			Protein	Nutrients (total)	Fat	Protein	Nutrients	
			Kg.	*Kg.*	*Kg.*			
1	Oct. 22, 1920	Oct. 28, 1920	1.037	7.34	0.231	91.0	90.7	Nearly adequate ration containing casein and lactalbumin.
2	Oct. 29, 1920	Nov. 13, 1920	.514	7.34	.231			Sucrose substituted for the casein and lactalbumin.
3	Nov 14, 1920	Nov. 27, 1920	1.037	7.34	.231			Nearly adequate ration containing casein and lactalbumin.
EXPERIMENT VI.—COW 246. QUANTITY OF PROTEIN INCREASED, QUALITY IMPROVED								
1	Dec. 2, 1922	Dec. 9, 1922	0.620	7.25	0.257	63.8	98.9	Ration deficient in protein.
2	Dec. 10, 1922	Dec. 22, 1922	1.195	7.25	.257			Casein put in ration in place of dextrine.
3	Dec. 23, 1922	Feb. 21, 1923	1.468	7.29	.246			Further addition of casein.
4	Feb. 22, 1923	Mar. 13, 1923	1.321	7.88				Cow put on ordinary grain mixture, silage, and alfalfa.
EXPERIMENT VII.—COW 246. CHANGE IN QUALITY OF PROTEIN, NO CHANGE IN QUANTITY								
1	Sept. 4, 1922	Sept. 13, 1922	1.230	7.27	0.209	97.3	83.6	Casein-corn-protein ration.
2	Sept. 14, 1922	Sept. 28, 1922	1.225	7.29	.277			Corn-protein ration.
EXPERIMENT VIII.—COW 423. CHANGE IN QUALITY OF PROTEIN, NO CHANGE IN QUANTITY								
1	Nov. 5, 1921	Nov. 12, 1921	0.775	5.67	0.229	103.3	100.2	Corn-protein ration.
2	Nov. 13, 1921	Nov. 23, 1921	.775	5.66	.134			Gelatin-corn-protein ration.
3	Nov. 24, 1921	Dec. 5, 1921	.775	5.66	.134			Milk-protein and corn-protein ration.

TABLE II.—*Quantities of separate feeds offered daily* [a]

EXPERIMENT I.—COW 17. (WEIGHT 375 KG.)

Period No.	Corn starch; corn dextrine	Sucrose	Casein	Lactalbumin	Gelatin	Corn meal	Corn gluten feed	Cottonseed meal	Linseed meal	Hominy meal	Wheat bran	Ground oats	Alfalfa hay	Timothy hay	Corn silage	$CaCO_3$	$Na_2HPO_4.12H_2O$	NaCl	Remarks
	Kg.	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	
1								0.292	0.292	1.169	0.780	1.949	1.81		11.34			0.059	Adequate ration.
2								.146	.146	.585	.390	.974	.91		11.34			.029	Energy and protein reduced.
3								.292	.292	1.169	.780	1.949	1.81		11.34			.059	Adequate ration.

EXPERIMENT II.—COW 54. (WEIGHT 472 KG.)

1	0.769		0.448	0.075		2.55					1.09			2.72	10.89			0.051	Adequate ration.
2			.590	.098		1.61					.69			2.04	8.16			.051	Energy reduced.
3	.769		.448	.075		2.55					1.09			2.72	10.89			.051	Adequate ration.

EXPERIMENT III.—COW 422. (WEIGHT 334 KG.)

1						3.953	1.438	0.450	0.225						9.07	0.091	0.354	0.067	Adequate ration.
2	1.132	0.453				3.800		.226	.113						9.07	.098	.531	.067	Quantity of protein reduced.
3						3.953	1.438	.450	.225						9.07	.091	.354	.067	Adequate ration.

EXPERIMENT IV.—COW 246. (WEIGHT 610 KG.)

1						0.33	4.62							1.81	13.61	0.049	0.360	0.176	Adequate ration.
2	0.575					3.17	.91							1.81	13.61	.058	.637	.188	Quantity of protein reduced.

[a] All except the hay and silage were fed as a concentrate mixture.

7582—25†—3

TABLE II.—*Quantities of separate feeds offered daily* [a]—Continued

EXPERIMENT V.—COW 54. (WEIGHT 484 KG.)

Period No.	Corn starch; corn dextrine	Sucrose	Casein	Lactalbumin	Gelatin	Corn meal	Corn gluten feed	Cottonseed meal	Linseed meal	Hominy meal	Wheat bran	Ground oats	Alfalfa hay	Timothy hay	Corn silage	$CaCO_3$	$NaH_2PO_4.12H_2O$	NaCl	Remarks
	Kg.	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	
1	0.769	------	0.448	0.075	------	2.55	------	------	------	------	1.09	------	------	2.72	10.89	------	------	0.051	Adequate ration.
2	.769	0.523	------	------	------	2.55	------	------	------	------	1.09	------	------	2.72	10.89	------	------	.051	Quantity and quality of protein reduced.
3	.769	------	.448	.075	------	2.55	------	------	------	------	1.09	------	------	2.72	10.89	------	------	.051	Adequate ration.

EXPERIMENT VI.—COW 240. (WEIGHT 596 KG.)

1	0.575	------	------	------	------	3.17	0.91	------	------	------	------	------	------	1.81	13.61	0.058	0.637	0.188	Protein inadequate.
2	------	------	0.575	------	------	3.17	.91	------	------	------	------	------	------	1.81	13.61	.058	.637	.188	Protein increased in quantity and quality.
3	------	------	.869	------	------	2.86	.91	------	------	------	------	------	------	1.81	13.61	.059	.642	.190	Further increase in protein.
4	------	------	------	------	------	2.16	------	1.078	0.539	------	1.62	------	2.72	------	13.61	------	------	.054	Ordinary ration.

EXPERIMENT VII.—COW 246. (WEIGHT 582 KG.)

1	1.140	------	0.565	------	------	0.97	1.82	------	------	------	------	------	------	1.81	13.61	0.057	0.596	0.187	Casein-corn-protein ration.
2	------	------	------	------	------	.33	4.62	------	------	------	------	------	------	1.81	13.61	.049	.360	.176	Corn-protein ration.

EXPERIMENT VIII.—COW 423. (WEIGHT 307 KG.)

1	------	------	------	------	------	2.65	2.28	------	------	------	------	------	------	9.07	0.073	0.230	0.056	Corn-protein ration.
2	1.480	0.456	------	------	3.88	1.13	.97	------	------	------	------	------	------	9.07	.076	.395	.061	Gelatin-corn-protein ration.
3	1.480	.456	0.320	0.068	------	1.13	.97	------	------	------	------	------	------	9.07	.076	.395	.061	Milk-corn-protein ration.

[a] All except the hay and silage were fed as a concentrate mixture.

DISCUSSION

It will be convenient to consider the data in these experiments in the following order:

1. Changes in the composition of the blood produced by the changes in the rations.
2. Changes in the yield and composition of the milk produced by the changes in the rations.
3. Physiological relations between the composition of the blood and the secretion of milk.

CHANGES IN THE COMPOSITION OF THE BLOOD

An inspection of Tables III to X will readily give some idea of the concentrations of plasma amino N that may occur under normal conditions. There are 11 determinations in these experiments made with cows that had been fed ordinary feeding materials, containing approximately the quantity of protein, and from 85 to 102 per cent of the energy, which would be required according to the Savage standard, the feeding being continued for a sufficient length of time to give an idea of the normal plasma amino N under these conditions. The figures varied from 2.20 to 2.64 mg. per 100 c. c. of blood plasma.

Figure 1 shows the changes in plasma amino N that occurred as a result of

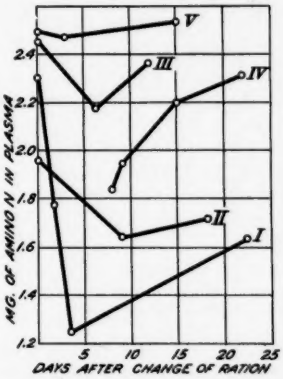

FIG. 1.—Effect on plasma amino N of reducing the protein (50 per cent) or energy content (33 per cent), or both, in the ration of a milking cow after a period on an adequate ration. Except in Experiment IV, the initial determinations give the level of plasma amino N in the first periods just before the rations were changed

Experiment I.—Energy and protein content of ration reduced

Experiment II.—Energy content of ration reduced

Experiment III.—Amount of protein reduced; no change in quality

Experiment IV.—Amount of protein reduced; no change in quality. No amino N determinations were made before the change of ration. This curve, like the others, shows a tendency for concentration of amino N to return to normal even while ration is still inadequate

Experiment V.—Quantity and quality of protein reduced

TABLE III.—*Experiment I, cow 17. Results of changing the energy content and the quantity of protein in the ration*

Period No.	Date	Milk (daily)					Blood		
		Yield	Nitrogen		Fat		Amino N*		Plasma lipoid phosphorus (mg. per 100 c. c.)
							Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	
	1920	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*			
1	May 1–10	12.85							
1	May 11	12.93	0.5045	65.23	3.43	443.9	4.10	2.30	7.8
2	May 12	12.25	.4922	60.28					
2	May 13	10.71	.5211	55.78	4.04	432.8	3.74	1.77	
2	May 14	9.89	.4964	49.08	3.88	384.1			
2	May 15	9.89	.4911	48.56	3.93	388.7	3.23	1.25	7.0
2	May 16–18	9.89							
2	May 19	10.30	.4676	48.15	3.48	358.7			
2	May 20–27	8.84							
2	May 28	9.21	.4738	43.63	3.32	304.1			
2	May 29–June 1	8.47							
2	June 2	7.94	.4600	36.30	3.62	284.3			
2	June 3	8.16					3.03	1.63	
2	June 4–6	7.76							
2	June 7	7.80	.4848	37.83	3.61	281.4	3.05	1.66	6.3
3	June 8	8.26							
3	June 9	10.84	.5292	57.13	3.81	423.3			
3	June 10	9.43					2.75	1.57	
3	June 11	9.30			3.94	366.4			
3	June 12–23	9.25							
3	June 24	9.03					3.63	2.64	

* Average difference in duplicate amino-N determinations, in blood 9.2 per cent, and in plasma 9.8 per cent.

reductions in these rations in the second periods of Experiments I to V. The curve in Experiment I shows that a sharp reduction in both protein and energy content of the ration of a milking cow produces a decided and abrupt drop in plasma amino N. In four days it dropped from 2.3 to 1.25 mg. per 100 c. c., or 46 per cent.

Experiment II shows that a similar drop in plasma amino N occurs when the energy content of the ration is reduced without materially altering the dietary protein. In period 1 of this experiment the cow was just recovering from the effects of a previous experiment, and the plasma amino N of 1.95 mg. per 100 c. c. was low. The energy content of the ration (Table I) was relatively higher than in Experiment I. Yet the plasma amino N dropped to 1.64 mg. per 100 c. c., or 16 per cent.

In Experiment II determinations were made of the glucose in the blood and plasma in order to determine whether this blood constituent underwent changes parallel to the changes in animo N. The results indicate that this was not the case; apparently the sugar content of the blood samples was more affected by the disturbance caused to the animal in obtaining them than by the changes in the diet. (See Table IV and its footnotes.)

TABLE IV.—*Experiment II, cow 54. Change in energy content of ration*

Period No.	Date	Milk (daily)							Blood			
		Yield	Nitrogen		Fat		Lactose		Amino N [a]		Sugar	
									Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1920	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*				
1	Nov. 25	11.66										
1	Nov. 26	11.34	0.4837	54.85	3.44	390.1	5.23	593.1				
1	Nov. 27	11.84	.4946	58.55	3.64	430.9	5.34	632.2	4.05	1.95	64.1	77.1
2	Nov. 28	11.11										
2	Nov. 29	11.34	.4598	52.14	3.77	417.5	5.39	611.2				
2	Nov. 30	10.52										
2	Dec. 1	11.16	.4539	50.65	3.39	378.3	5.42	628.2				
2	Dec. 2	11.16	.4714	52.68	3.37	376.1						
2	Dec. 3–4	11.09										
2	Dec. 5	10.71	.4592	49.16	3.49	373.6	5.37	574.9				
2	Dec. 6	10.52	.4661	49.05	3.37	354.7	5.39	567.2				
2	Dec. 7	10.93							3.87	1.64	63.5	70.9
2	Dec. 8	10.39	.4419	45.91	3.47	360.5						
2	Dec. 10	10.57	.4547	48.06	3.61	381.5						
2	Dec. 11	10.61	.4502	48.01	3.43	364.1						
2	Dec. 12	10.80	.4646	50.16	3.49	376.8						
2	Dec. 13	10.30	.4569	47.04	3.20	329.5						
2	Dec. 15	10.39	.4424	45.95	3.21	333.5						
2	Dec. 16	9.35							3.72	1.72	[b] 73.6	[b] 89.8
2	Dec. 17	9.34										
2	Dec. 18–24	9.66										
3	Dec. 25	9.33										
3	Dec. 26	9.66										
3	Dec. 27	10.61										
3	Dec. 28	10.61										
3	Dec. 29–31	10.48										
	1921											
3	Jan. 1–7	10.52										
3	Jan. 8	10.93	.4702	51.40	3.40	371.7						
3	Jan. 9	10.66			3.39	361.4						
3	Jan. 10	10.30	.4675	48.14	3.40	350.1						
3	Jan. 11	9.98			3.43	342.3						
3	Jan. 12	10.43	.4772	49.79	3.86	402.7						
3	Jan. 13	9.71			3.51	340.7						
3	Jan. 15	9.89	.4819	47.65	3.51	347.1			4.14	2.47	[c] 54.7	[c] 61.3

[a] Average difference in duplicate amino-N determinations in blood 3 per cent, and in plasma 4.3 per cent.

[b] This cow was most disturbed in obtaining this blood sample.

[c] This cow was least disturbed in obtaining this blood sample.

TABLE V.—*Experiment III, cow 422. Change in the quantity of protein in the ration; no change in its quality*

Period No.	Date	Milk (daily)					Amino N[a]	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1921	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*		
1	Sept. 16–21	8.65	5.01	433	0.643	55.6	[b] 4.22	[b] 2.45
2	Sept. 22 [c]							
2	Sept. 25	7.12	4.80	342	.613	43.7		
2	Sept. 26	6.99	4.98	348	.613	42.2		
2	Sept. 27	6.76	4.81	325	.625	42.2		
2	Sept. 28	6.12	4.74	290	.643	39.3	3.76	2.17
2	Sept. 29	6.31	4.65	293	.620	39.1		
2	Sept. 30	6.49	4.55	295	.623	40.4		
2	Oct. 1	5.85	5.38	315	.628	36.7		
2	Oct. 2	5.72	4.78	273	.600	34.3		
2	Oct. 3	5.67	5.03	285	.660	37.4		
2	Oct. 4 [c]						3.76	2.36
2	Oct. 6	5.53	5.77	320	.636	35.2		
2	Oct. 7	5.31	5.57	296	.640	34.0		
2	Oct. 8	5.44	5.30	288	.644	35.0		
2	Oct. 10 [c]							
3	Oct. 11 [c]	5.67	4.96	281	.644	36.5		
3	Oct. 12	6.40	5.03	322	.606	38.8		
3	Oct. 13	6.80	6.14	418	.643	43.7		
3	Oct. 14	6.89	4.83	333	.679	46.8	4.73	
3	Oct. 15	6.58	5.83	383	.705	46.4		
3	Oct. 16	6.99	5.94	415	.712	49.8		
3	Oct. 17	6.89	5.94	410	.722	49.8	4.36	2.33
3	Oct. 18	6.99	5.90	412	.710	49.6		
3	Oct. 19	7.21	5.77	416	.676	48.8		
3	Oct. 20	7.22	5.92	427	.682	49.2		
3	Oct. 21	7.26	5.74	417	.732	53.2		
3	Oct. 22	7.08	6.45	456				
3	Oct. 25	7.54	6.35	478	.722	54.4		
3	Oct. 26	7.12	6.25	445	.750	53.4	4.63	2.36
3	Oct. 27	6.85	6.33	433	.748	51.2		
3	Oct. 28	7.44					3.94	2.20
3	Oct. 29	7.12	6.24	444	.729	51.9		

[a] Average difference in duplicate amino-N determinations in blood 4.9 per cent, and in plasma 3.1 per cent.

[b] Sample taken Sept. 19.

[c] Feed refused or withheld: Sept. 22, grain 2.01 kg.; Oct. 4, silage 1.97 kg. and grain 1.38 kg.; Oct. 10, grain 1.1 kg.; Oct. 11, grain 3.43 kg.

In Experiment III, in which the quantity of dietary protein in the ration was reduced without change in its quality[4] or in the energy content of the ration, there was initially a slight drop in plasma amino N. In period 1 it was 2.45 mg. Six days after the change of ration it was 2.17 mg., or 11.4 per cent lower. This is hardly considerable.

A comparison of the curves in Experiments I and III shows plainly that the cut in the energy along with the protein in Experiment I increased decidedly the abruptness and magnitude of the drop in plasma amino N. From this and from Experiment II it is evident that a *sharp cut (33 per cent) in the energy content of the ration of a milking cow brings about a decided reduction in plasma amino N. The energy content of the ration is an important factor in determining the level of plasma amino N.*

The curve in Experiment V shows that no change in plasma amino N occurred when the quality as well as the quantity of the dietary protein was reduced. In period 1 the plasma amino N was 2.43 and 2.55 mg., and in period 2 it was 2.47 and 2.53 mg. This result, compared with that in Experiment III, suggests that the quality of the dietary protein may affect the level of plasma amino N. (See later.) Experiments III and V indicate that *a sharp cut of 50 per cent in the dietary protein with a milking cow often does not*

[4] By quality here we mean the amino acid composition of the mixture of dietary proteins. Wherever used with reference to proteins in this paper, it is used in this sense. By a protein of good quality we mean one that because of its amino acid composition can be used efficiently in the synthesis of milk and tissue proteins.

greatly affect the level of the plasma amino N.

In Experiment IV the amount of protein was reduced without change in its quality and without change in the energy content of the ration. No determinations of the amino N were made during the period on an adequate ration. The concentration of amino N in the blood plasma on the eighth day of the second period is decidedly lower than the average concentration of amino N found in these experiments for cows on adequate rations; but this may be partly due to the low energy content of this cow's ration. (See Table I.)

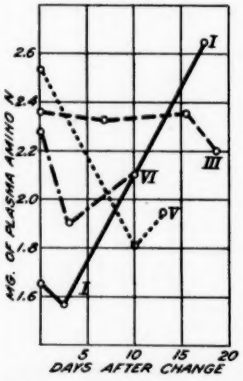

FIG. 2.—Effect on plasma amino N of an increase of 75 to 100 per cent in the amount of protein or 50 per cent in the energy content, or both, in the ration of a milking cow after a period on a ration inadequate in these constituents. The first determinations in this chart show the level of the plasma amino N just before the rations were increased

Experiment I.—Amount of energy and protein in ration increased

Experiment III.—Amount of protein increased; no change in quality

Experiment V.—Amount and quality of protein increased

Experiment VI.—Amount and quality of protein increased

NOTE.—Experiment II does not appear in this chart because no determination of amino N was made in Experiment II until 21 days after adequacy of ration was restored. (See Table V.)

After the initial drop in plasma amino N in Experiments I, III, and IV there was a subsequent rise. In Experiment I the plasma amino N on May 15 was 1.25 mg. per 100 c. c. On the same ration on June 3 and 7 it was 1.63 and 1.66 mg., respectively, or 32 per cent higher. In Experiment IV it rose from 1.84 mg. on November 13 to 2.28 mg. on December 9, or 24 per cent. The rise in Experiment III was inconsiderable, 8.7 per cent.

Figure 2 shows the changes in plasma amino N that occurred when the protein or the protein and energy content of the rations were again increased. In Experiment III, in which the quantity of the dietary protein was increased 91 per cent without change in its quality, there was no change in plasma amino N. In Experiments V and VI, in which the quality of the dietary protein was improved along with a similar increase in its quantity (102 per cent in the former experiment and 92 per cent in the latter), the plasma amino N actually dropped decidedly. In Experiment V it dropped from 2.53 mg. to 1.81 mg., or 28 per cent, and in Experiment VI from 2.28 mg. to 1.9 mg., or 17 per cent. From these data the following conclusions may be drawn:

1. *When a milking cow has been on an inadequate protein ration for some time, the quantity of the dietary protein may be increased 90 to 100 per cent without causing an increase in plasma amino N. The latter may even drop decidedly.*

2. *The quality of the dietary proteins under certain conditions affects decidedly the plasma amino N, and the ration containing the more efficient protein mixture is associated with the lower level of plasma amino N.*

3. *The level of amino N associated with a given ration depends largely upon the nutritional condition of the animal as affected by dietary history.* This is shown best by comparing periods 1 and 3 of Experiments I and V (Tables III and VII). In period 1 of Experiment V the plasma amino N was 2.49 mg. In the first determination of period 3 on the same ration it was 1.81 mg. The corresponding data for Experiment I are 2.3 mg. and 1.57 mg., respectively.

When the quantities of the protein in the rations in Experiments V and VI were increased, at least two factors probably contributed to the drop in plasma amino N that occurred: (1) The improvement in the quality of the dietary protein, and (2) the nutritional condition of the animal which was produced by the preceding period of reduced feeding.

In Experiment I, in which the energy content of the ration was increased simultaneously with the quantity of protein, the plasma amino N apparently at first did not change. At the end of period 2 it was 1.66 mg. Three days after the change of ration it was 1.57 mg. Later it rose to 2.64 mg. without further change of ration. The increase in the energy content of the ration in this experiment much more

TABLE VI.—*Experiment IV, cow 246. Quantity of protein in ration reduced. No change in quality*

Period No.	Date	Milk (daily)					Amino N[a]		Tryptophane			
									Blood		Plasma	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	Individual determinations (mg. per 100 c. c.)	Average (mg. per 100 c. c.)	Individual determinations (mg. per 100 c. c.)	Average (mg. per 100 c. c.)
	1922	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*						
1	Oct. 27-31	10.16										
1	Nov. 1	10.34							1.03,1.04,1.00	1.02		
1	Nov. 2, 3	10.10										
1	Nov. 4, a. m.	9.84										
2	Nov. 4, p. m.											
2	Nov. 5	9.43										
2	Nov. 6	8.98										
2	Nov. 7	8.57										
2	Nov. 8	8.62										
2	Nov. 9	8.30										
2	Nov. 10	8.57										
2	Nov. 11	8.21	4.31	355	0.546	44.9						
2	Nov. 13	8.48	3.88	329	.514	43.6		1.84			1.00,1.08	1.04
2	Nov. 14	8.62	3.87	334	.528	45.5	4.58	1.95	1.456, 1.464	1.46		
2	Nov. 15-19	8.97	3.86	346	.548	49.2		2.20	1.03, 1.05	1.04		
2	Nov. 20	8.39	3.89	326	.555	46.6						
2	Nov. 23-26	7.78	3.92	305	.553	43.0						
2	Nov. 27	7.26	4.04	293	.550	39.9	4.67	2.31				
2	Nov. 30-Dec. 4	7.54	3.86	291	.552	41.5						
2	Dec. 6	7.62	3.62	276	.543	41.3	[b]2.67	2.89	0.92, 0.73	.82		
2	Dec. 7-8	7.94	4.02	319	.544	43.2						
2	Dec. 9	7.76	3.91	303	.544	42.2	3.93	2.28	0.64, 0.62	.63		

[a] Average difference in duplicate amino-N determinations in blood 7.9 per cent, and in plasma 6.1 per cent.
[b] This unusual blood amino-N is substantiated by four determinations made by two different methods. It is difficult to see how it could be due to accident in analytical work.

TABLE VII.—*Experiment V, cow 54. Change in the quantity and quality of protein in ration*

Period No.	Date	Milk (daily)							Amino N[a]		Sugar	
		Yield	Nitrogen		Fat		Lactose		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1920		*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*				
1	Oct 22-25	12.84										
1	Oct. 26	13.25	0.4965	65.76	3.49	462.3			4.01	2.43		
1	Oct. 27	13.06	.4864	63.54	3.58	467.7						
1	Oct 28	12.97	.4988	64.72	3.48	461.5			4.34	2.55		
2	Oct 29	12.47	.4843	60.42	3.46	431.6	5.28	658.6				
2	Oct. 30	12.57	.4659	58.54	3.46	434.8						
2	Oct. 31	12.25	.4499	55.10	3.34	409.1	5.28	646.6				
2	Nov. 1	11.79	.4600	54.25	3.22	379.8						
2	Nov. 2	10.93	.4545	49.68	3.21	350.9			4.06	2.47		
2	Nov. 3	12.07	.4478	54.04	2.94	354.7	5.23	631.1				
2	Nov. 4	10.52	.4346	45.74	3.12	328.4						
2	Nov. 5	10.80	.4059	43.82	3.06	330.4						
2	Nov. 7	10.89	.4699	51.15	3.43	373.9	5.36	583.5				
2	Nov. 8	11.52										
2	Nov. 9	11.78	.4467	52.63	2.91	342.9						
2	Nov. 11	12.11	.4409	53.40								
2	Nov. 12	11.20	.4499	50.40	2.89	323.8	5.39	603.9				
2	Nov. 13	11.30							4.42	2.53		
3	Nov. 14	11.30										
3	Nov. 16	11.34	.4767	54.06	3.01	341.3	5.39	611.2				
3	Nov. 17	12.16	.4565	55.49	3.08	374.4						
3	Nov. 18	12.25	.4709	57.67	3.06	374.8						
3	Nov. 19	12.66	.4748	60.09	3.11	393.6						
3	Nov. 21-22	12.45										
3	Nov. 23	12.61	.4672	58.92	3.20	403.5	5.23	659.5				
3	Nov. 24	12.02	.4489	53.96	3.39	407.5			3.63	1.81	64.0	75.5
3	Nov. 25	11.66										
3	Nov. 26	11.34	.4837	54.85	3.44	390.1	5.23	593.1				
3	Nov. 27	11.84	.4946	58.55	3.64	430.9	5.34	632.2	4.05	1.95	64.1	77.1

[a] Average difference in duplicate amino N determinations in blood 3.4 per cent, and in plasma 9.3 per cent.

than offset the increased output of energy due to the rise in milk yield which took place in the third period. The data here, therefore, exclude the increased energy requirement for the increased milk yield in Experiments V and VI as a considerable factor tending to effect the drop in plasma amino N produced by increasing the dietary protein.

From the data in Figures 1 and 2 it appears that with milking cows, where absorption is a fairly constant and continuous process, and where there is a constant demand for energy for milk secretion, the plasma amino N is more affected by (1) the energy content of the ration, (2) the quality of the dietary protein, and (3) the nutritional condition of the animal than it is by the actual amount of protein in the ration. Increases in the quantity of dietary protein are not infrequently followed by decreases in the concentration of the plasma amino N, and it seems a rather general rule that a lower level of plasma amino N is associated with a higher efficiency of the dietary protein for milk secretion.

It must be remembered that all of these experiments were made on milking animals, and it may be pointed out that a low plasma amino N associated with a high efficiency of dietary protein is what one would expect in such animals on the supposition that the efficiency for milk secretion of the plasma mixture of amino acids varies in the same direction as the efficiency of the dietary protein. When the efficiency for milk secretion of the plasma amino acid mixture was high, the mammary gland would take out a large proportion of the total amino N for the synthesis of the milk proteins, and less would be left in the circulation. The results suggest, therefore, that the quality as well as the quantity of the plasma amino acid mixture is subject to variation.

TABLE VIII.—*Experiment VI, cow 246. Increase in quantity and quality of protein in ration*

Period No.	Date	Milk (daily)					Amino N [a]		Tryptophane	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c.c.)	Plasma (mg. per 100 c.c.)	Individual determinations (mg. per 100 c.c.)	Average (mg. per 100 c.c.)
	1922	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*				
1	Dec. 2–8	7.72	3.84	297	0.546	42.1				
1	Dec. 9	7.76	3.91	303	.544	42.2	3.93	2.28	0.64, 0.62	0.63
2	Dec. 10									
2	Dec. 11	7.98	3.76	300	.554	44.2				
2	Dec. 13	8.48	3.88	329	.551	46.8	3.93	1.90	.69, .59	.64
2	Dec. 14	8.53	3.91	333	.549	46.8				
2	Dec. 15–19	7.64	4.02	298	.569	42.2				
2	Dec. 20	7.44	3.96	295	.588	43.8	4.01	2.10	1.01, .96, 1.03	1.00
2	Dec. 22	7.94	3.97	315	.564	44.8				
3	Dec. 23–28	7.99	3.90	318	.558	45.5				
3	Dec. 29 [b]									
	1923									
3	Jan. 7	7.51	3.92	302	.577	43.3				
3	Jan 8	6.53	3.88	271	.604	39.5	4.06	2.03	.65, .62	.63
3	Jan. 9–18	6.73	4.03	271	.607	40.9				
3	Jan. 27–Feb. 5	6.62	4.08	269	.600	39.9				
3	Feb. 6	6.80	4.00	272	.596	40.6	4.14	2.26	.83, .80, .74	.79
3	Feb. 9–15	6.93	3.93	268	.581	40.3				
3	Feb. 16	7.35	4.17	306	.614	45.1	3.63	1.98	.95, .86	.90
3	Feb. 18–21	6.89								
4	Feb. 22–28	6.81								
4	Mar. 1–6	7.32								
4	Mar. 7–12	7.05								
4	Mar. 13	7.39					3.83	2.22	1.201, 1.205	1.20

[a] Average difference in duplicate amino-N determinations in blood 4.5 per cent, and in plasma 3.3 per cent.

[b] Off feed. Refused 1.66 kg. of grain on Dec. 30; 2.29 kg. of grain on Dec. 31; and 2.37 kg. of grain, 1.18 kg. of hay, and 5.91 kg. of silage on Jan. 1.

In order to test this hypothesis, the free tryptophane and the total amino N of the blood have been followed simultaneously in Experiments IV, VI, and VIII. (See Tables VI, VIII, and X.) From the results of these experiments the following conclusions may be drawn:

1. *Under appropriate conditions very great changes may occur in the concentration of the free tryptophane in the blood while at the same time relatively little or no change occurs in the blood amino N.* In Experiment IV the tryptophane in the blood on November 14 was 1.46 mg. per 100 c. c., and on December 9 it was 0.63 mg., a difference of 57 per cent. The difference in blood amino N between these dates was only 14 per cent. In Experiment VI the blood tryptophane increased from 0.63 to 1.2 mg., or 90 per cent, with no increase in blood amino N; and in Experiment VIII the blood tryptophane increased about 100 per cent between periods 1 and 3 with practically no change in blood amino N.

2. *These alterations in the composition of the blood mixture of amino acids may, under certain conditions, continue for weeks.* On the low-protein ration in period 3 of Experiment IV the blood tryptophane dropped from 1.46 mg. to 1.04, 0.82, and 0.63 mg. in four consecutive samples taken during a period of 25 days. This drop in blood tryptophane was probably caused by a decrease in the rate at which the tissues yielded tryptophane to the blood.

3. *These experiments show that changes occur in the composition of the blood mixture of amino acids (a) when the quantity of protein in the ration is reduced, (b) when the quality of the dietary protein is changed, and (c) when the quantity of protein in the ration is increased.*

The rise in blood tryptophane between periods 1 and 2 from 1.03 to 1.46 mg. in Experiment IV, and from 0.34 to 0.45 mg. in Experiment VIII, might well be expected even in view of the fact that the quantities of tryptophane in the protein of the rations were low in the first periods of both experiments and still lower in the second periods. An amino acid like valine, which is lower relatively in both muscle and corn proteins than in milk proteins and is absent from gelatin, might well determine the extent of increase in negative N balance in changing from the rations of the first period to those of the second period, and thus might effect a rise in blood tryptophane. This explanation accords with that given above for the subsequent drop in blood tryptophane in period 2 of Experiment IV.

TABLE IX.—*Experiment VII, cow 246. Change in quality of protein in ration; no change in quantity*

Period No.	Date	Milk (daily)					Amino N [a]	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1922	*Kg.*	*Per cent*	*Grams*	*Per cent*	*Grams*		
1	September 4–8	12.59	3.41	420	0.440	54.5		
1	September 9	12.70	3.67	466	.440	56.0	3.98	
1	September 10–12	11.90	3.78	454	.447	53.6		
1	September 13 [b]	11.79	3.71	436	.456	53.8	3.66	1.83
2	September 14	12.11	3.86	468	.453	54.9		
2	September 15	11.93	3.63	433	.438	52.3		
2	September 16	11.61	3.40	395	.438	50.9		
2	September 17	11.66						
2	September 18	11.52	3.98	459	.462	53.2		
2	September 19	11.52	4.04	465	.456	52.5	4.37	2.43
2	September 20	11.52	3.83	441	.451	52.0		
2	September 21	11.48	3.86	443	.435	49.9		
2	September 22	11.39	3.82	435	.453	51.6		
2	September 23	11.30	4.28	483	.459	51.8		
2	September 24	11.48	4.06	466	.471	54.0		
2	September 25	11.25	3.93	442	.473	53.2		2.08
2	September 26	11.20	4.17	467	.497	55.6		
2	September 27	11.34						
2	September 28	10.89	3.78	412	.502	54.6		

[a] Average difference in duplicate amino-N determinations in blood 2.9 per cent, and in plasma 2.9 per cent.

[b] On Sept. 13, 0.45 kg. of grain was refused

As a matter of convenience, the qualitative changes in the mixture of amino acids in the whole blood rather than in the blood plasma have been studied. The conclusions, that have been reached, however, are undoubtedly applicable to the blood plasma as well.

TABLE X.—*Experiment VIII, cow 423. Change in quality of protein in ration; no change in quantity*

Period No.	Date	Milk (daily)					Amino N*		Tryptophane (mg. per 100 c. c.)
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	
	1921	*Kg.*	*Per cent*	*Gm.*	*Per cent*	*Gm.*			
1	Nov. 5, 6	7.01	5.51	386	0.626	43.8			
1	Nov. 8	7.21	5.57	402	.632	45.6	4.37	2.46	0.34
1	Nov. 10–11	6.94	5.77	400	.625	41.0			
1	Nov. 12	6.89	5.73	395	.651	44.9	4.20	2.24	
2	Nov. 13	6.89	5.75	396	.647	44.6			
2	Nov. 14	6.35	5.62	357	.679	43.2			
2	Nov. 15	6.26	4.98	312	.672	42.1			
2	Nov. 17	5.72	6.59	376	.738	42.2			
2	Nov. 18	5.40	6.55	354	.719	38.8			
2	Nov. 19	5.26	6.19	326					
2	Nov. 20	5.76	7.44	429					
2	Nov. 21	5.44	6.21	338	.692	37.7			
2	Nov. 22	5.44	6.04	328	.672	36.5	4.22	2.37	.45
2	Nov. 23	5.22	6.42	335	.688	35.9			
3	Nov. 24	5.49	6.22	341	.658	36.1			
3	Nov. 25	5.90	5.96	351	.623	36.7			
3	Nov. 26	6.21	5.43	338					
3	Nov. 27	6.49	5.07	329	.637	41.3			
3	Nov. 28	6.49	5.69	369	.631	40.9			
3	Nov. 29	6.26	5.02	314					
3	Nov. 30	6.26	5.02	314					
3	Dec. 1	6.44	5.07	326	.638	41.1	4.14	2.28	.66
3	Dec. 3	6.53	5.35	350					
3	Dec. 4	5.94	5.35	318	.626	37.2			
3	Dec. 5	5.44	5.54	302	.653	35.6	4.57	3.08	.69

* Average difference in duplicate amino-N determinations in blood 2.9 per cent, and in plasma 1.6 per cent.

Although it was found, as was to have been expected, that a high plasma amino N is, in general, associated with a dietary protein mixture of low efficiency, there are a number of circumstances in which this might not be true. Thus in Experiments VII and VIII (Tables IX and X) in changing from the first to the second period the quality of the dietary protein was reduced with no change in quantity; the milk yield was reduced; the concentrations of certain amino acids in the blood plasma were reduced, whereas the concentrations of others were increased; and, whether the total plasma amino N would increase or not, one can readily see, would largely depend upon the responsiveness of the mechanism destroying these latter amino acids to the increases in their concentrations. In Experiment VII, where the energy of the ration and the plasma amino N were low, the total amino N rose when the ration was changed; but in Experiment VIII, where the energy of the ration and the plasma amino N were higher, the total amino N did not rise. In fact, there was no significant change in plasma amino N throughout the latter experiment until after the corn-milk protein ration of period 3 had been fed for a considerable time. The abrupt rise from 2.28 to 3.08 mg. per 100 c. c. at this time probably marks a change in the storage capacity of the tissues.

CHANGES IN THE YIELD AND COMPOSITION OF MILK

Figure 3 shows that in Experiments I to V, when the protein or energy, or both, in the rations were reduced, the milk yields dropped decidedly; figure 4 shows that this effect on the milk yields was reversed when these dietary changes were reversed; and Figure 5 shows that the milk yields varied with the quality of the dietary protein in Experiments VII and VIII, in which the quality of the dietary protein was changed without change in its quantity or in the energy of the ration.

Figures 6 to 13 show the changes in the composition of the milk in all ex-

cept Experiment IV, where no determinations were made in period 1. The results may be summarized as follows:

1. Experiments II and V show that the concentration of lactose in the milk of an individual cow is exceedingly constant and is unaffected by wide variations either in the quantity and quality of the dietary protein or in the quantity of carbohydrate in the ration. It varied in these two experiments from 5.23 per cent to 5.42 per cent, or 3.6 per cent difference.

2. A reduction in the energy content of the ration, either alone (Experiment II) or together with the protein (Experiment I), has no constant tendency to reduce the concentration of milk fat, but does effect a reduction in the concentration of milk N. In Experiment I the concentration of milk fat was higher on the ration low in its protein and energy content than on the original ration.

3. A reduction in the quantity (Experiment III) or quantity and quality (Experiment V) of the dietary protein, without altering the energy content of the ration, reduces the concentrations of milk N and fat.

4. In Experiments VII and VIII, where only the quality of the dietary protein was changed, the concentrations of milk N and fat were higher when the dietary protein was qualitatively less well adapted to the secretion of the milk proteins.

5. In all these experiments where only the protein of the ration was altered there is in general a parallelism between the changes in the concentration of milk N and fat that is certainly quite evident.

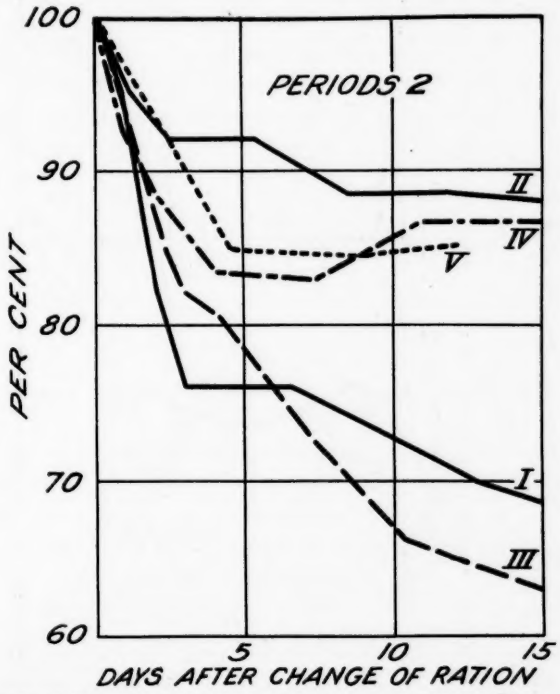

FIG. 3.—Experiments I–V. Change of milk yield when amount of protein or energy, or both, were reduced in the ration after period on adequate ration. The yield before the ration was changed is taken as 100 per cent

RELATION BETWEEN THE COMPOSITION OF BLOOD AND THE SECRETION OF MILK

The results of these experiments show, as was to have been expected, that the relations between the diet, the composition of the blood, and the secretion of milk are exceedingly complicated. We are still very far

from knowing all the factors which affect these relationships, and the physiological conclusions to be drawn from such experiments as those which have just been presented must, therefore, be more or less tentative for a long time to come. But the manner in which diet affects the composition by additional work more extensive than can be carried out by a single investigator. In the meantime it is worth while to set forth some of the conclusions which appear to be reasonably justified, though perhaps not fully established, by the work done so far.

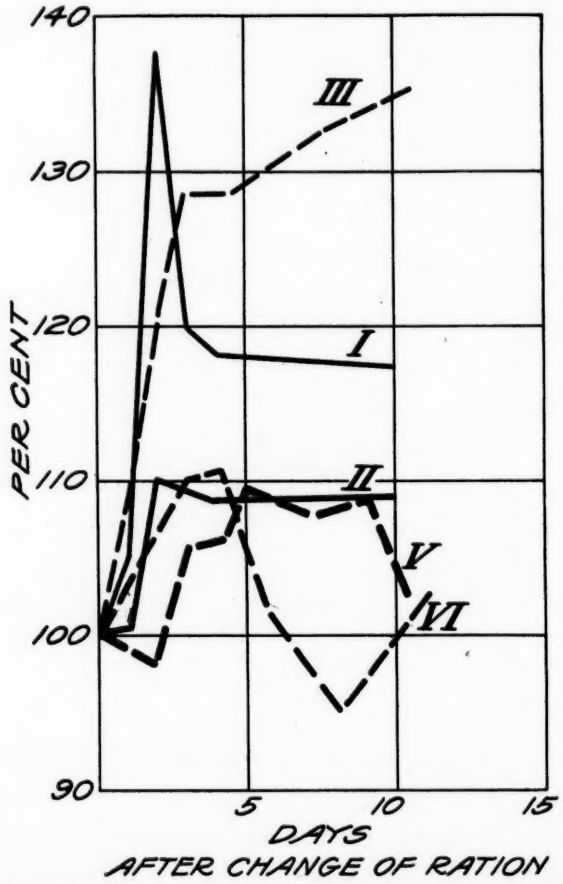

FIG. 4.—Experiments I, II, III, V, and VI. Change of milk yield when amount of protein or energy, or both, were increased in ration after a period of reduced feeding. Yields at end of previous period taken as 100 per cent

of the blood and in which this again affects the various bodily activities are matters of such fundamental physiological importance that it will be profitable to determine and record the facts in this field even though their full significance cannot be realized until they are supplemented

In order to interpret the results which are to be considered in this section, it is necessary to bear in mind that the lactose, fat, and proteins of milk are made in the mammary gland from glucose, phosphatide, and amino acids, respectively, that are taken from the plasma of the blood.

It is necessary also to bear in mind certain evidence, which will be reviewed briefly at this point, that indicates that the secretion of milk may be affected through either the precursor of milk fat or that of milk protein. This evidence gives some idea of the differences in the changes that occur in the composition of milk when its secretion is affected by these different precursors.

Morgen and a number of his collaborators have conducted an extensive series of experiments on sheep and goats in which the effects on milk yield of varying the protein and fat of the diet were studied (*13–16*). The plan of the experiments was to change the rations by substituting protein or fat for an isodynamic amount of carbohydrate, or vice versa, and then, after an interval of some days, to determine the effect which had been brought about on the yield and composition of milk. It was found that milk yield could be affected by such changes in either the protein or fat of the ration. Changes in the dietary protein affected the milk yield through a wide range in the level of the protein feeding; changes in the dietary fat, on the other hand, only when they were made at low levels of fat feeding. Changes in the dietary protein produced only irregular changes in the composition of the milk; changes in the dietary fat, when they produced changes in milk yield at all, generally changed the concentration of milk fat in the same direction as the change made in the dietary fat; and the concentration of milk nitrogen, in the opposite direction. It seems likely that the changes in the composition of milk produced by changes in the quantity of dietary fat would have been even more regular than they were had they been determined immediately instead of after an interval of some days. The concentration of lactose in the milk was not significantly affected by any of the dietary changes studied in Morgen's experiments.

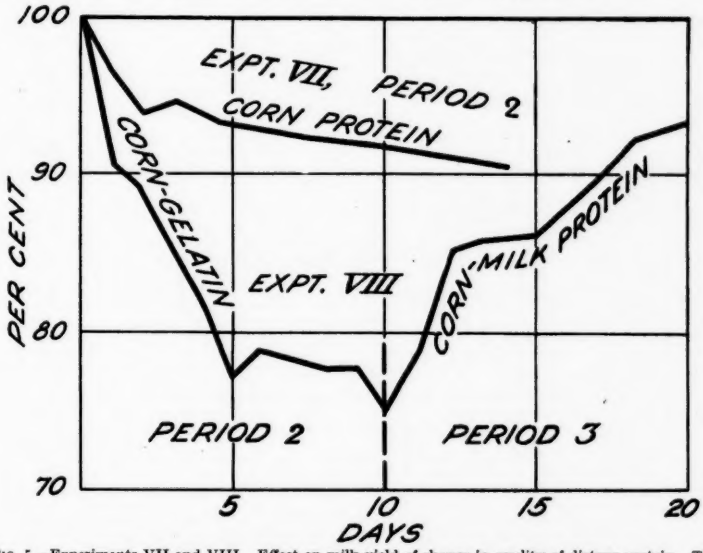

FIG. 5.—Experiments VII and VIII. Effect on milk yield of change in quality of dietary protein. The yield before the ration was changed is taken as 100 per cent

Subsequent to Morgen's work, an extensive series of experiments, in which the effects of dietary changes in fat on the yield and composition of cow's milk were studied, was made by the Deutscher Landwirtschaftsrat (*4*). The results of these experiments indicate that the dietary fat has less effect on milk secretion in cows than it has in sheep and goats. But an experiment reported by Jordan and Jenter (*9*) indicates that, under appropriate circumstances, milk secretion in cows may be affected by changes in dietary fat and that, when it is so affected, the concentration of milk nitrogen varies in a direction opposite to that of the change made in the dietary fat.

Experiments reported in the present article and discussed to some extent in the preceding pages show that milk secretion in cows is markedly affected by changes in the dietary protein.

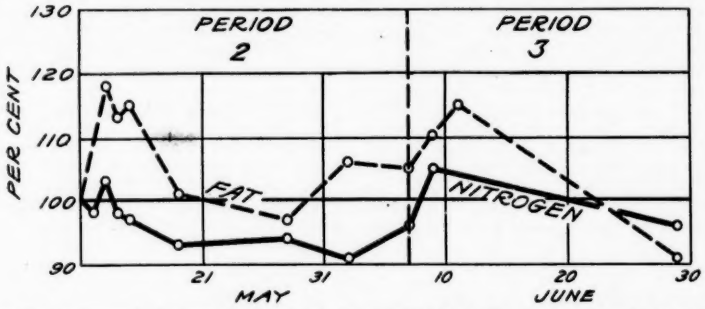

FIG. 6.—Experiment I. Changes in concentration of nitrogen and fat of milk. The concentration in period 1 is taken as 100 per cent. In period 2 the protein and energy of the ration were reduced, and in period 3 they were restored to adequacy

In general, it may be said that milk secretion may be affected by changes in either the fat or protein of the ration, but much more easily by changes in the protein. When the milk yield is affected by changes in the dietary fat, there is generally a tendency for the concentration of milk nitrogen to vary in a direction opposite to that of the change made in the dietary fat, while this is not the case when milk yield is affected by changes in the dietary protein. The situation is most easily accounted for by supposing that a change in the protein of the ration tends to affect milk secretion through a change in the precursor of milk protein—namely, the free amino acids of the blood—while a change in the dietary fat tends to affect it through a change in the precursor of milk fat—namely, the phosphatide of the blood. These considerations justify the attempt to interpret the results which are to be discussed in this section on the hypothesis that changes in diet frequently affect milk yield through changes brought about in the free amino acids of the blood.

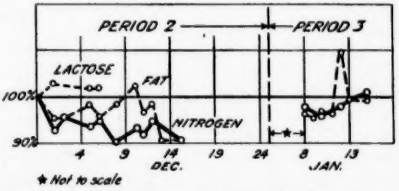

FIG. 7.—Experiment II. Changes in the composition of milk. The concentration in period 1 is taken as 100 per cent. In period 2, the energy content of the ration was reduced, while the protein was left unchanged; in period 3, the energy was restored to adequacy

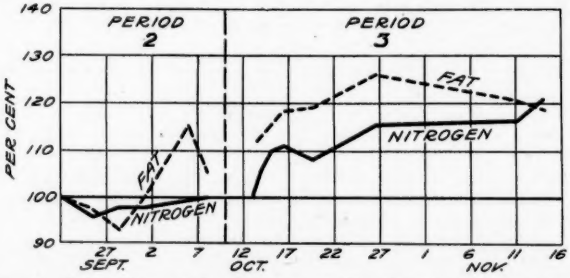

FIG. 8.—Experiment III. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2, the quantity of protein in the ration was reduced without change in its quality; in period 3, it was restored to adequacy

The discussion of the individual experiments of this series may well be begun with that of Experiment II, in which the quantity of energy (chiefly carbohydrate) in the ration was markedly reduced without making any con-

siderable change in the protein. As a result there was a drop in the concentration of amino N in the blood and plasma, a drop in milk yield, and a drop in the concentration of milk N (figs. 1, 2, and 7). When the energy of the ration was restored to the initial level, the amino N of the blood and plasma, the milk yield, and the concentration of milk N all rose (figs. 4 and 7). The amino N in the blood and plasma was determined so long after the second change in ration that its rise has not been shown in any of the figures, but it may be seen in Table IV. The results of this experiment may be taken as particularly strong and consistent evidence for the view that the free amino acids of the blood play a predominant part in regulating milk secretion. This is rather surprising when we bear in mind that only the energy content of the ration in this experiment was changed.

In Experiments III and IV the quantity of dietary protein was reduced without change in its quality and without change in the dietary energy; and in Experiment III the protein was after a time restored to the initial level. In Experiment I the dietary protein and energy were reduced together and later restored to the initial level. In all three of these experiments the milk yield and the concentration of milk N followed the changes in dietary protein in the same sense in which they followed the dietary energy in Experiment II (figs. 3, 4, 6, and 8). In all three cases, also, there is reason to think that the reduction in dietary protein was followed by a reduction in the level of the plasma amino N (fig. 1). These results are therefore like those of Experiment II, consistent with the view that the dietary changes affected milk yield through the free amino acids of the blood.

In all three of these experiments, however, the plasma amino N began to rise while the cows were still on the inadequate rations and while the milk yields in two of the cases were still falling off (figs. 1 and 3). Further, when the rations were restored to the initial levels in Experiments I and III, there was a marked tendency for the milk yields and the concentration of milk N to rise in both cases, though in neither case was there any tendency for the plasma amino N to rise.

The results of Experiments V and VI how even more clearly that there is no hard and fast relation between the rate of milk secretion and the concentration of total amino N in the blood plasma. In these experiments both the quantity and the quality of the dietary protein were changed. When the quantity and quality of the dietary protein were reduced together, there was a decided tendency for the milk yield to fall off and for the concentration of milk N to be reduced; and both these tendencies were reversed when the quantity and quality of the dietary protein were increased (figs. 3, 4, 9, and 10). But reduction in the quantity and quality of the dietary protein caused no significant change in the level of plasma amino N (fig. 1); and the opposite changes in the dietary protein were followed in both experiments by decided reductions in the level of plasma amino N (fig. 2) in spite of the rising milk yields and rising concentrations of milk N.

The lack of parallelism between plasma amino N and milk yield, which is to some extent apparent in all the

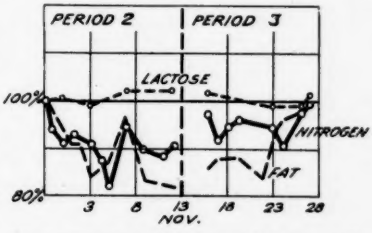

FIG. 9.—Experiment V. Changes in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the protein of the ration was reduced in both quantity and quality; in period 3 it was restored to adequacy

experiments, but particularly in experiments V and VI, is no real reason for abandoning the hypothesis that in these experiments the milk yield was controlled through the plasma amino-acid mixture. The manner in which milk yield may be increased by a change in the plasma amino-acid mixture, even though the concentration of total plasma amino N may be at the same time reduced, is shown by the results of Experiments IV, VI, and VIII, in which the plasma amino N and the free tryptophane of the blood were followed simultaneously. (Tables VI, VIII, and X.) These experiments have already been discussed to some extent. They show that when the quantity of protein in the ration is reduced, the composition of the blood mixture of amino acids may be very considerably altered; that when the milk yield falls off rapidly during a period on a ration inadequate in protein, and the plasma amino N at the same time gradually rises, the concentration of tryptophane in the blood may constantly fall; that when

the dietary protein is increased and the milk yield increases without a rise in total plasma amino N, the concentration of blood tryptophane may rise; and, finally, that when the milk yield is altered by a change in the quality of the dietary protein, the concentration of blood tryptophane may be very greatly altered, although the total plasma amino N may be unchanged. The correspondence between the changes of diet on the one hand and of the yield and composition of milk on the other, taken along with these results which show that the quality of the plasma amino acid mixture may vary as well as its quantity, justify the belief that the changes in dietary protein in these experiments influenced the yield of milk largely through changes in the quality of the plasma amino-acid mixture.

In Experiments VII and VIII the quality of the dietary protein was changed without change in its quantity. protein without changing the total energy of the ration, there was a strong tendency for the concentrations of milk N and fat to run parallel (figs. 8 to 12). There is no reason to think that changes in dietary fat had any influence on the concentration of milk fat in these experiments, for, although small changes were frequently made in the dietary fat at the same time that the protein was altered, there was no tendency toward parallelism between changes in dietary fat and changes in milk fat. In addition, the experiments of the Deutscher Landwirtschaftsrat (*4*) referred to above show that the changes which were made in the dietary fat in these experiments were not of such a nature that they could have had any influence either on the secretion of milk fat or on milk secretion in general. They also indicate that it was not through changes in the amount of fat formed from protein or through the phosphatide of the blood plasma that

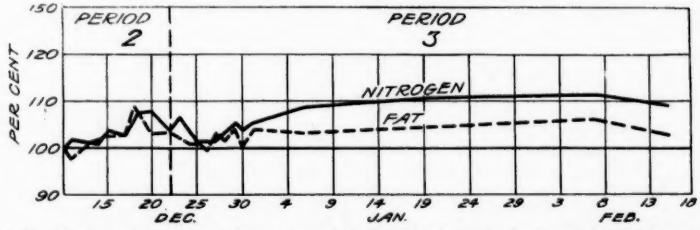

FIG. 10.—Experiment VI. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the quantity of protein in the ration was increased and its quality improved; in period 3, the quantity and quality of the ration were still further increased

The resulting changes in plasma amino N and in total milk yield are in fairly close agreement with the conclusions which have been drawn from the other experiments, and no special comment is required. But the concentration of milk N decreased in these two experiments when the quality of the dietary protein was improved, and vice versa, and this is not in entire accord with the results of the other experiments. The factors which control the concentration of milk N are, however, in all probability numerous and complicated, and it is not felt that the lack of parallelism between quality of dietary protein and concentration of milk N in these two experiments is sufficient seriously to impair the force of the conclusions which have been drawn from the others.

This part of the discussion may be concluded by pointing out certain other interesting aspects of the results. When changes in milk secretion were brought about by altering the dietary the changes in dietary protein affected the secretion of milk fat. The results, therefore, indicate that the secretion of milk fat may be influenced through the amino-acid mixture of the blood. It must be pointed out, however, that in Experiment I, where the protein and energy of the ration were changed together, and in Experiment II, where the energy was changed while the protein was kept constant, there was no marked tendency toward parallelism between the concentrations of milk protein and milk fat. Considerable changes in the concentration of plasma amino N had here no tendency to affect the secretion of milk fat. It seems not unreasonable to suppose that reductions in the energy of a ration tend to cause body fat to be thrown out into the blood and that this increased supply of fat in the blood may overcome the tendency for the concentration of milk fat to go down with that of the milk nitrogen. This is suggested by the work of Eckles and

Palmer (*5*), in which they found that when the protein and energy of the ration of milking cows were reduced as in Experiment I the composition of the milk fat approached that of body fat, and further, by the relatively small drop in plasma phosphatide as compared with that of the plasma amino-N in Experiment I.

Another interesting indication which is given by the results of Experiments I, II, and III as shown in Figures 1 and 3 is that reductions in dietary protein tend chiefly to reduce the efficiency for milk secretion of the plasma amino-acid mixture, while reductions in dietary energy tend to reduce the quantity of plasma amino N and perhaps, at the the same time, to increase the inefficiency for milk secretion of the plasma amino-acid mixture. In Experiment I the dietary protein and energy were reduced together, there was a very large reduction in plasma amino N, and a large reduction in milk secretion. In Experiment III the dietary protein alone was reduced, there was a small reduction in plasma amino N, but a reduction in milk secretion even a little larger than in Experiment I. In Experiment II the dietary energy alone was reduced, the plasma amino N fell far below the normal level, while the reduction in milk secretion was quite small. It seems difficult to explain these relations, except on the supposition that the efficiency for milk secretion of the plasma amino-acid mixture was much higher in Experiments I and II, where the dietary energy was reduced, than in Experiment III, where the protein was reduced while the energy was kept constant.

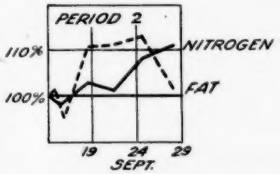

FIG. 11.—Experiment VII. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the quality of the protein fed was not so good as in period 1; its quantity was unchanged

SUMMARY

In experiments on milking cows, changes have been made in the energy and protein of the rations both separately and simultaneously. The protein has been changed in quantity or quality, or both. The effects of these changes on the composition and yield of milk, on the concentration of amino N of the blood and plasma, and, in some cases, on the concentration of free tryptophane and other constituents in the blood, have been determined. The results obtained may be summarized as follows:

1. A sharp cut in the energy content of the ration of a milking cow produces a decided reduction in the plasma amino N; with milking cows the energy content of the ration is an important factor in determining the level of plasma amino N.

2. A sharp cut in the quantity of protein in the ration produced only a slight drop in plasma amino N when the quality of the protein was unchanged, and no drop at all when the quality of the dietary protein was reduced along with the quantity.

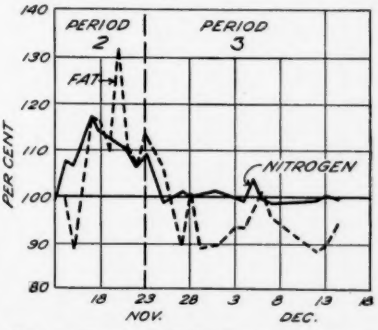

FIG. 12.—Experiment VIII. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the quality of the protein in the ration was decreased without change in its quantity; in period 3 the quality was increased above its original level

3. When a milking cow has been for a time on a ration inadequate in protein, the quantity of protein may be increased 90 to 100 per cent without an increase in plasma amino N. If the quality of the dietary protein is improved simultaneously with the increase in its quantity, the plasma amino N drops decidedly.

4. Under appropriate conditions the quality of the dietary protein affects decidedly the level of plasma amino N and the ration containing the more efficient protein mixture is associated with the lower level of plasma amino N.

5. The nutritional condition of an animal, as brought about by its dietary history, has a very great effect upon the level of plasma amino N associated with a given ration.

6. In the case of milking cows, where absorption is a fairly constant and con-

tinuous process and where there is a constant demand for energy for milk secretion, the level of plasma amino N is frequently more influenced by (a) the energy content of the ration, (b) the quality of the dietary protein, and (c) the nutritional condition of the animal than by the actual quantity of protein in the ration.

7. Under appropriate conditions very great changes may occur in the concentration of the free tryptophane of the blood, while at the same time little or no change occurs in the level of total amino N. These alterations in the composition of blood mixture of amino acids may continue for weeks. They occur when either the quantity or quality of the dietary protein is changed.

8. Sharp reductions in the quantity of energy in the ration or in either the quantity or quality of the protein are immediately followed by reductions in milk yield. The composition of the milk also undergoes changes which may reasonably be attributed to the changes in diet. When the original ration is again substituted for the reduced ration, there is a general tendency for the yield and composition of the milk to return to their original status. A study of these changes in the rations and milk, along with those occurring simultaneously in the amino N, tryptophane, and other blood constituents which have been determined in these experiments, justifies the working hypothesis that changes in dietary protein and energy affect milk secretion largely by inducing changes in the quantity and quality of the amino-acid mixture circulating in the blood plasma.

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